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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

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INVENTOR(s)/APPLICANT(s)

LAST NAME	FIRST NAME	MIDDLE NAME	RESIDENCE (CITY AND EITHER STATE OR FOREIGN COUNTRY)
DOU	SHENSHEN		CARMEL, INDIANA
SONG	HO	YEONG	CARMEL, INDIANA
GLASEBROOK	ANDREW	LAWRENCE	ZIONSVILLE, INDIANA
NOBLITT	TIMOTHY	WAYNE	INDIANAPOLIS, INDIANA
NA	SONGQING		CARMEL, INDIANA

TITLE OF THE INVENTION (280 characters max)

METHOD OF TREATING ACUTE RESPIRATORY DISTRESS SYNDROME (ARDS) USING FLINT POLYPEPTIDES

CORRESPONDENCE ADDRESS

Eli Lilly and Company
Patent Division/D.C. 1104
Lilly Corporate Center
Indianapolis, Indiana 46285

STATE	IN	ZIP CODE	46285	COUNTRY	USA

ENCLOSED APPLICATION PARTS (*check all that apply*)

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The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

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Respectfully submitted,
SIGNATURE Anne I. Craig Date 12 / 22 / 98

TYPED or PRINTED NAME ANNE I. CRAIG REGISTRATION NO. (*if appropriate*) 32,976

[ ] Additional inventors are being named on separately numbered sheets attached hereto

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METHOD OF TREATING ACUTE RESPIRATORY DISTRESS SYNDROME (ARDS) USING FLINT POLYPEPTIDES

BACKGROUND OF THE INVENTION

This invention relates to methods preventing or treating conditions caused or exacerbated by apoptosis inflammation. Of particular importance is apoptosis induced by Fas ligand (FasL) and Fas receptor (Fas) binding (also referred to as FasL-Fas binding). Also of particular importance are methods of preventing or treating conditions caused by a proinflammatory response caused or exacerbated by FasL induced neutrophil activation. One example of a condition caused or exacerbated by the cellular pathway induced by FasL and Fas binding is acute respiratory distress syndrome (ARDS). ARDS is often encountered with other more serious illnesses, for example, sepsis. There are estimated to be 150,000 cases of ARDS annually in the US, and up to an additional 200,000 cases outside the US, for a world patient population of approximately 350,000. ARDS carries a high mortality rate and approximately 30% of the patients die of the disease in the ICU.

This invention further relates to the discovery that FLINT (Fas ligand inhibitor) polypeptide binds preferentially to FasL. This invention still further relates to the novel method of modulating the deleterious effects of FasL-Fas binding, for example, FasL induced apoptosis and/or FasL induced inflammation by administering polypeptides of the present invention. Accordingly, when FLINT polypeptide is administered to a patient in need of modulation of the deleterious effects of FasL-Fas binding, FLINT polypeptide is capable of preventing or treating disease, in particular ARDS.

FasL (also called CD95L and APO1L) is expressed on various cell types and can produce a biological responses such as proliferation, differentiation, immunoregulation, inflammatory response, cytotoxicity, and apoptosis. Interestingly, mutations in FasL, the ligand for the TNFR-family receptor FAS/APO (Suda et al., 1993, Cell 75:1169-78, are associated with autoimmunity (Fisher et al., 1995, Cell 81:935-46), while overproduction of FasL may be implicated in drug-induced hepatitis.

Apoptosis plays a central role in both development and in homeostasis. Cells die by apoptosis in the developing embryo during morphogenesis or synaptogenesis and in the adult animal during tissue turnover or at the end of an immune response. Because the physiological role of apoptosis is crucial, aberration of this process can be detrimental. For example, unscheduled apoptosis of certain brain neurons contributes to disorders such as Alzheimer's and Parkinson's disease, whereas the failure of dividing cells to initiate apoptosis after sustaining severe DNA damage contributes to cancer.

Survival signals from the cell's environment and internal sensors for cellular integrity normally keep a cell's apoptotic machinery in check. In the event that a cell loses contact with its surroundings or sustains irreparable damage, the cell initiates apoptosis. A cell that simultaneously receives conflicting signals driving or attenuating its division cycle also triggers apoptosis. Mammals have evolved yet another mechanism that enables the organism actively to direct individual cells to self-destruct. This kind of "instructive" apoptosis is important especially in the immune system. Death receptors-cell surface receptors that transmit apoptosis signals initiated by specific "death ligands" - play a central role in instructive role apoptosis. These receptors can activate death caspases within seconds of ligands binding, causing an apoptotic demise of the cell within hours.

Death receptors belong to the tumor necrosis factor (TNF) receptor gene superfamily, which is defined by similar, cysteine-rich extracellular domains. The death receptors contain in additional a homologous cytoplasmic sequence termed the "death domain". Death domains typically enable death receptors to engage the cell's apoptotic machinery, but in some instances they mediate functions that are distinct from or even counteract apoptosis.

Fas (also called CD95 or Apo1) is a well-characterized death receptor. Fas and Fas ligand (FasL) play an important role in apoptosis. Fas L is a homotrimeric molecule. It is suggested that each FasL timer binds three Fas molecules. Because death domains have a propensity to associate with one another, Fas ligation leads to clustering of the receptors' death domains. An adapter protein called FADD (Fas-associated death domain; also called Mort1) then

binds through its own death domain to the clustered receptor death domains. FADD also contains a "death effector domain" that binds to an analogous domain repeated in tandem within the zymogen form of caspase-8 (also called FLICE, or MACH). Upon recruitment by FADD, caspase-8 oligomerization drives its activation through self-cleavage. Caspase-8 then activates downstream effector caspases such as caspase-9 committing the cell to apoptosis. Ashkenazi A., et al. "Death Receptors: Signaling and Modulation SCIENCE 281, 1305-1308 (August 1998).

Although it triggers apoptosis in T lymphocytes, FasL is also proinflammatory. FasL has been shown to stimulate neutrophil, also called polymorphonuclear leukocytes (PMNs), activation. (Chen J. et at, Science 282: 1714-17 (1998)) FasL-Fas binding has been implicated in clonal deletions of autoreactive lymphocytes in peripheral lymphoid tissues and in elimination of autoreactive lymphocyte populations, thus contributing to homeostatsis of the immune system. However, it has been found that expression of FasL on myotubes or pancreatic islets of transgenic mice induces a granulocytic response that accelerates graft rejection (Allison J. et al., Proc. Natl. Acad. Sci, 94:3943-47 (April 1997); Kang S-M. et al., Nature Medicien, Vol. 3, No. 7, 738-743 (July 1997)).

As mentioned above, at least one of the effects of FasL-Fas receptor binding is apoptosis, which is necessary for homeostatsis. However, sometimes the balance of ligand-receptors binding is upset in stress, disease or trauma. One of the negative effects of unregulated FasL-Fas binding is runaway apoptosis. Another effect of said binding is the destruction of cells caused by neutrophils that have been activated by FasL.

One of the more tragic outcomes of runaway apoptosis and inflammation is ARDS or, if multiple organs are involved, sepsis. ARDS is most often encountered with other serious illnesses. Thirty-eight percent (38%) of ARDS cases occur in sepsis patients. At the present time there is no FDA approved pharmacological treatment for ARDS. Experimental therapies include corticosteroids, ventilator therapy (PEEP), surfactant replacement therapy and inhaled nitric oxide therapy. None of these therapies have proven efficacious.

ARDS and sepsis are characterized by an overactivation of cytokine pathways where there is massive apoptosis and/or inflammation of cells in lungs and multiple organs, respectively. Sepsis can be found in acute bacterial infections, in particular, gram negative bacterial infections.

ARDS and sepsis, which often develops into life-threatening shock, are systemic clinical situations caused by toxic substances released from microorganisms during severe infection. In humans, ARDS and sepsis are commonly caused by endotoxins secreted from gram-negative bacteria. Septic shock is characterized by a drastic fall in blood pressure, cardiovascular collapse and multiple organ failure, and is responsible for over 100,000 deaths a year in the US alone. In the past 10 years, mortality in patients with sepsis has only slightly decreased, despite aggressive intensive care treatment. An entire medical specialty, called Critical Care Medicine, has developed around the septic patient, delivering hemodynamic, metabolic, ventilative and renal support. Yet, mortality of septic shock patients remains high at 35-45% even in the most sophisticated medical centers of the world.

Thus, compounds that bind FasL, especially those compounds that selectively bind FasL, can interfere with FasL binding to Fas receptor. Conditions caused or exacerbated by aberrant FasL binding to the Fas receptor can be controlled by such compounds.

It is an object of the present invention to identify compounds that interact and/or bind between Fas L.

It is another object of the present invention to identify the biological effects of compounds that interact and/or bind FasL.

It is yet another object of the present invention to identify, prevent and/or treat conditions caused or exacerbated by undesired interactions and/or binding between FasL and its respective receptor(s).

It is a still further object of the present invention to provide novel methods of modulating interactions and/or binding between FasL and its receptor(s).

It is yet another object of the present invention to treat and/or prevent ARDS by administering effective amounts of FLINT polypeptides.

SUMMARY OF THE INVENTION

Disclosed are methods for modulating selected TNFR interactions with their respective TNFR family ligands comprising administering an effective amount of FLINT polypeptide. Also disclosed are methods for treating a patient in need of FLINT polypeptide activity comprising administering to said patient an effective amount of a FLINT polypeptide the FLINT polypeptide is selected from the group consisting of a FLINT polypeptide analog, a biologically active FLINT polypeptide fragment, a FLINT polypeptide fusion protein and a FLINT polypeptide isoform. FLINT

polypeptide activity is binding of FasL that modulates FasL induced apoptosis and/or FasL mediated inflammation. Thus, FLINT is capable of preventing or treating ARDS. Further, FLINT is capable of binding LIGHT, another TNFR family ligand. LIGHT is involved in cell proliferation and regulation of LIGHT mediated cell proliferation is possible. Also disclosed are methods of modulating TNFR interactions with their respective TNFR ligands in cells selected from the group consisting of T cells, myocytes, renal tubule epithelial cells (RTC), neutrophils, neurons, thyrocytes, stroma cells, acinar cells, Sertoli cells, macrophages, hepatocytes, leukemia cells, cells of the kidney cortex and tumor cells, preferably neutrophils, T cells, epithelial cells and macrophages.

DETAILED DESCRIPTION OF THE INVENTION

This invention relates to the discovery that FLINT (Fas ligand inhibitor) polypeptide binds to FasL. Further, this binding is preferential and specific. This invention further relates to the novel method of modulating the deleterious effects of FasL-Fas binding, for example, FasL induced apoptosis and/or FasL induced inflammation, by administering an effective amount FLINT polypeptides. Accordingly, when FLINT polypeptide is administered to a patient in need of FLINT polypeptide activity, for example, modulation of the deleterious effects of FasL-Fas binding, FLINT polypeptide is capable of preventing or treating disease.

Fas is a type I membrane bound receptor that initiates an apoptotic signal when bound to its ligand, FasL or agonistic antibodies. Under normal conditions, Fas is ubiquitously expressed.

FasL is a 40 kDa type II membrane protein belonging to the TNF family. FasL can induce apoptosis of Fas-expressing cells, whether as a membrane-bound form or as a 17 kDa soluble form which is released by metalloprotease-mediated proteolytic shedding. The functional soluble form is responsible for killing Fas-sensitive cells through either autocrine suicide or paracrine death of neighboring cells, whereas the membrane form requires direct cell contact to mediate cytolysis (DeMaria R., et al, Immunol. Today 19:21, March 1998). FasL is expressed in immune-privileged tissues of the eye, testis, brain and some tumors. It has also been found in kidney and lung as well as in activated thymocytes, splenocytes, and T lymphocytes.

The present invention related, in one aspect, to FLINT polypeptides which are capable of binding to the Fas ligand protein and thereby mediating or modulating the activity of FasL especially where FasL is involved in binding Fas. Thus, FLINT polypeptides are capable of modulating or mediating downstream effects of FasL including, but not limited to, apoptosis and/or inflammation. Due to the unique ability of FasL-Fas interaction to cause cell death, aberration of the function of this interaction can be deleterious to the organism. Both excessive and deficient function of FasL can contribute to the pathological manifestations of various diseases. Finding ways to modulate the function of FasL constitutes a potential clue for new therapeutic approaches to diseases. In view of the important role of FasL-Fas, it seems particularly important to design drugs that can block the cytotoxic function of FasL, possibly by binding of FLINT to FasL or otherwise inhibiting the interaction between FasL and Fas under those conditions in which enhance FasL-mediated apoptosis.

By employing methods for identifying compounds that bind Fas ligand, applicants have discovered that human FLINT polypeptides are capable of disrupting FasL-Fas receptor. Applicants have discovered methods for modulating the TNFR proteins and their respective ligand interactions where such interactions cause or exacerbate disease and methods for preventing or treating diseases. If specific interactions can be controlled, a variety of treatment possibilities become available.

It has been discovered that FLINT polypeptides bind to FasL with at least the same, if not greater, affinity than the Fas receptor itself. Thus, by binding FasL, FLINT polypeptides can interfere with FasL binding to Fas receptor and interfere with events downstream of such binding. At least one of the downstream effects of FasL-Fas receptor binding is apoptosis. As mentioned earlier, one of the negative effects of unregulated FasL-Fas binding is runaway apoptosis. One of the more tragic outcomes of runaway apoptosis is ARDS. ARDS is most often encountered in patients with other serious, acute injuries and illnesses. For example, burns, cardiopulmonary bypass, multiple fractures, pancreatitis, prolonged hypotension, sepsis, toxic inhalation, and trauma are major risk factors. ARDS is most often encountered in an acute care setting such as the medical or surgical intensive care units in patients with other major illnesses. Sepsis as used herein is intended to include systemic inflammatory-response syndrome (SIRS), sepsis, severe sepsis, septic shock, and septic shock syndrome. Sepsis can be found in acute bacterial infections, in particular, gram negative bacterial infections.

Another downstream effect of FasL-Fas receptor binding is neutrophil activation wherein cells are destroyed by neutrophils activated by FasL. By antagonizing FasL, FLINT polypeptides can modulate the destruction of healthy cells caused by neutrophils activated by FasL. Recently, it was discovered that FasL induces in peritoneal exudate cells (PEC) the processing and release of IL-1β that is responsible for the neutrophil infiltration. (Miwa K., et al. Nature Medicine, 4, #11: 1287-1292 (Nov. 1998)). Miwa et al. found that inoculation of tumor cells expressing Fas ligand into wild-type mice induces a massive neutrophil infiltration that is, in contrast, suppressed in IL-1αβ knockout mice. This indicates that FasL has an inflammatory role. It also suggests that apoptosis may itself induce inflammation under certain conditions.

It is possible that FasL induced apoptosis and FasL mediated inflammation occurring singly, together and/or potentially in combination with additional mechanisms create substantial destruction of surrounding cells. Modulation of FasL would enable treatment of diseases and unhealthy conditions caused or exacerbated by any and all of these mechanisms.

FLINT is a newly identified member of the TNFR superfamily. The FLINT polypeptide of the present invention is soluble TNFR comprising extracellular domains. The nucleotide sequences and polypeptides described herein are referred to as "FLINT." FLINT polypeptide does not include any transmembrane domains and is, therefore, soluble. FLINT has also been called OPG3 (osteoprotegrin 3) or TNFRsol. FLINT is believed to be closely related to TNFR 6α and TNFR 6β discussed in WO98/30694 claiming priority to U.S.S.N. 60/035,496 and TR4 discussed in EP 0861850A1, the teachings

of which are incorporated herein by reference. Specifically, the present invention contemplates using nucleic acids (SEQ ID NO.:1) and polypeptides encoded thereby which are implicated in conditions including, but not limited to, sepsis, ARDS, multiple organ failure and/or other conditions where there is massive inflammation and/or cells undergo massive apoptosis or destruction.

The present invention provides methods of preventing or treating conditions involving the use of isolated FLINT nucleic acid compounds and FLINT polypeptides functionally related to the tumor necrosis factor receptor (TNFR) superfamily.

In one embodiment, the present invention relates to a method of preventing or treating conditions caused or exacerbated by FasL-Fas binding including FasL-mediated apoptosis and/or a proinflammatory response, more particularly, a proinflammatory response caused by FasL induced neutrophil activation. In normal liver and other organs, there is a low constitutive level of Fas expression. However, both Fas and FasL expression is upregulated under stress, including infection, disease and trauma. An example of a condition caused or exacerbated by destructive effects of FasL and Fas binding is ARDS and sepsis. ARDS and sepsis are commonly correlated. Thus, a discussion of sepsis is important for understanding the etiology of many cases of ARDS.

Sepsis can be found in acute bacterial infections, in particular, gram negative bacterial infections. In particular, the presence of endotoxin from Gram negative bacteria signals the innate immune system to upregulate bacterial clearance and/or killing mechanisms. The intensity of such responses contributes to septic shock.

Controlling the response to the endotoxin could decrease the inflammatory response. It is the bacterium's outer double-membrane coat consisting of a sugar-plus-fat molecule called lipopolysaccharide (LPS) that causes the cellular response.

Usually, the progression of sepsis into septic shock coincides with a rapid increase in circulating levels of inflammatory cytokines such as TNF-α, IL-1β, IL-8, and IL-6. The sudden increase in the concentration of these cytokines, also called a "cytokine storm", is believed to be the underlying reason for the onset of the shock. Support for the notion that cytokine "friendly fire" is a major factor responsible for the severity of sepsis and the likelihood of death, came from animal models showing that neutralizing antibodies to TNF-α, the first cytokine elaborated in the septic inflammatory cascade, prevented death in mice exposed to lethal injections of E. coli or endotoxin. (Beutler B. et al, Science 229: 869-871 (1985). Additional support came from studies showing that injections of either TNF-α or IL-1 mimic the physiological changes of septic shock, and that blocking IL-1 activity with IL-1 receptor antagonist (IL-1ra) was effective in protecting animals from lethal bacteremia or endotoxemia. These results strongly suggested that reducing the levels of circulating TNF-α and /or IL-1 can attenuate the progression of sepsis into septic shock and pointed toward the possibility that anti-cytokine therapy could be effective in reducing the risk of dying from septic shock. In clinical trials using anti-cytokine agents, (anti-IL-1ra or anti-TNF-α), the reduction in mortality has been low. However, the research has been important because information about the treatment window is emerging. It has been determined that the timing of initiation of treatment in sepsis is very important.

Similarly, ARDS is an acute, life threatening illness characterized by severe hypoxemia and lung infiltration due to capillary leakage in the alveoli. The underlying problem is disruption of the integrity of the alveolar-capillary barrier of the lungs, which results in leakage of fluid and proteins into the alveoli and prevents proper oxygenation of the venous blood. Furthermore there is degradation of the surfactant proteins that contributes to the surface tension that keeps the alveoli open. ARDS has a very high mortality with approximately one third of the patients succumbing to the disease, which is due in part to the complete lack of effective therapies.

The exact cause of ARDS is unknown but it appears to be triggered by trauma or systemic inflammatory responses. Normal alveoli are inflated and compliant allowing red blood cells to be oxygenated. Damaged alveoli are fluid-filled, collapsed and non-compliant, as a result of fluid and protein leaking through damaged capillary and alveolar membranes into the interstitial space and the alveoli themselves. Oxygenation is severely impaired. Hypoxia results as red blood cells shunt past damaged alveoli without becoming oxygenated. (Wiedemann and Tai, Cleve. Clinic J. Med. 64:366 (1997).

ARDS research efforts have focused on pro-inflammatory cytokines, specifically TNF-α, IL-1, IL-6, and IL-8, some of which are elevated during ARDS. Experimental treatments revolving around cytokine antagonism have included prostaglandin E1, anti-TNF, antioxidants, and antiproteases. Unfortunately, little or no benefit has been observed clinically from any experimental treatments to date.

Despite many attempts, ARDS mortality continues to be in the 30% range, with almost all deaths occurring within 30

days. The prognosis of the ARDS patient is related to the initiating event. For example, ARDS in sepsis patients has the worst prognosis, while patients with ARDS in a background of pancreatitis or fat embolism have the best prognosis.

Without intending to limit the invention, it is believed that cytokines such as TNFα are unleashed early but diminish over time. It is believed that FasL expression is upregulated at a later stage and continues to exacerbate the condition by recruiting neutrophils and inducing further apoptosis. Such timing leaves open a treatment window not available with anti-TNFα treatments, among others.

In sepsis, multiple organs fail, blood pressure falls drastically and the cardiovascular system collapses. FasL has been implicated in failure of multiple organs; kidney (Schelling, J.R., et al., "Fas-Dependent Fratricidal apoptosis Is A Mechanism of Tubular Epithelial Cell Deletion in Chronic Renal Failure" Laboratory Investigation 78 #7: 813-824 (1998); liver (Kondo, T. et al. "Essential Role of the Fas ligand in Development of Hepatitis", Nature Medicine 3 #4, 409-413 (1997)) and pancreas (DeMaria R. et al "Fas-FasL Interactions: A Common Pathogenic Mechanism In Organ-specific Autoimmunity" Immunol. Today 19:121 (March 1998), among others. FasL is naturally expressed on the vascular endothelium where it can induce apoptosis in Fas-expressing immune cells as they enter the cell wall. (Sata M., et al. J. Clin. Invest. 102:9, 1682-1689 (1998)).

Applicants disclose two different animal experiments showing that the administration of FLINT polypeptide is capable of blunting or eliminating the body's response to LPS. As demonstrated in the Examples below, FLINT polypeptide was effective in reducing the deleterious

effects of LPS (1) at different time periods, including well after anti-TNFα antibodies failed and (2) as effectively as anti-FasL antibody. In *in vivo* models, administration of FLINT offers protection in liver failure (Example 17) and protected against lethality in sepsis (Example 18). Example 19 discusses the use of FLINT polypeptides to treat or prevent ARDS. In a model of human ARDS, rabbits are challenged with hyperoxia to induce the ARDS symptomology and then treated with FLINT polypeptides and solute permeability across the alveolar epithelium is measured.

Patients suffering from these conditions are treated by administering an effective amount of FLINT polypeptides encoded by the isolated nucleic acid compound comprising SEQ. ID NO.: 1 or fragment thereof. Other preferred nucleic acid compounds comprise nucleotides 88-900 of SEQ ID NO:1 or nucleotides 102-536 of SEQ ID NO:1. The present invention relates to a method of administering polypeptide products produced by a nucleic acid that is at least 75% identical, and preferably at least 95% identical, to a nucleic acid that encodes SEQ ID NO:2, or fragment thereof as long as those products are capable of binding FasL. It is understood that nucleic acids that hybridize to the polynucleotide of SEQ ID NO:1, or fragments thereof, under low or high stringency conditions are intended to be included as long as such polynucleotides encode a polypeptide that is capable of binding FasL. Such binding is selective in that TNFα is not bound by FLINT.

In another embodiment the present invention relates to a method of preventing or treating conditions caused or exacerbated by FasL-Fas binding including FasL-mediated apoptosis and/or a proinflammatory response, more particularly, a proinflammatory response caused by FasL induced neutrophil activation. ARDS and

sepsis are examples such conditions. Patients suffering from sepsis are treated prophylactically with a pharmaceutical composition comprising FLINT polypeptides to prevent the onset of ARDS. Patients suffering from ARDS are treated by administering a pharmaceutical composition comprising an effective amount of FLINT polypeptides, or functional fragment thereof, wherein said polypeptide molecule comprises the sequence identified as SEQ ID NO:2. Examples of functional fragments of SEQ ID NO.: 2 include amino acid regions comprising residues 30-300 (SEQ ID NO.: 5), residues 34-195 (SEQ ID NO:6), residues 30-194 (SEQ. ID NO.:7), residues 72-151 (SEQ ID NO.:8) SEQ ID NO.:7 can be further subdivided into subregions 1 and 2. Subregion 1 is a 43 amino acid stretch comprising residues 72-114 inclusive(SEQ ID NO.:9). Subregion 2 is a 37 amino acid stretch comprising residues 115-151 inclusive(SEQ ID NO.:10).

This invention further provides a method for modulating selected TNFR interactions with their respective TNFR family ligands *in vitro* comprising administering an effective amount of the FLINT polypeptide.

This invention also provides a method for modulating selected TNFR interactions with their respective TNFR family ligands *ex vivo* comprising administering an effective amount of the FLINT polynucleotide, more particularly during gene therapy.

This invention further provides, among other things, a method for modulating FasL mediated apoptosis mediated cell proliferation *in vivo, in vitro and ex vivo.*

Still further the present invention provides methods of preventing or treating ARDS by administering pharmaceutical compositions comprising FLINT polypeptide.

DEFINITIONS

The terms "complementary" or "complementarity" as used herein refer to the capacity of purine and pyrimidine nucleotides to associate through hydrogen bonding to form double stranded nucleic acid compounds. The following base pairs are related by complementarity: guanine and cytosine; adenine and thymine; and adenine and uracil. As used herein, "complementary" means that the aforementioned relationship applies to substantially all base pairs comprising two single-stranded nucleic acid compounds over the entire length of said molecules. "Partially complementary" refers to the aforementioned relationship in which one of two single-stranded nucleic acid compounds is shorter in length than the other such that a portion of one of the molecules remains single-stranded.

"Conservative substitution" or "conservative amino acid substitution" refers to a replacement of one or more amino acid residue(s) in a polypeptide or peptide as stipulated in Table 1.

"Fragment thereof" refers to a fragment, piece, or sub-region of a nucleic acid or polypeptide molecule whose sequence is disclosed herein, such that the fragment comprises 5 or more amino acids, or 10 or more nucleotides that are contiguous in the parent polypeptide or nucleic acid compound. Fragment thereof may or may not retain biological activity. For example, a fragment of a polypeptide disclosed herein could be used as an antigen to raise a specific antibody against the parent polypeptide molecule. When referring to a nucleic acid compound, "fragment thereof" refers to 10 or more contiguous nucleotides, derived from the parent nucleic acid, and also, owing to the genetic code, to the complementary sequence.

For example if the fragment entails the sequence 5'-AGCTAG-3', then "fragment thereof" would also include the complementary sequence, 3'-TCGATC-5'.

The term "fusion protein" denotes a hybrid protein molecule not found in nature comprising a translational fusion or enzymatic fusion in which two or more different proteins or fragments thereof are covalently linked on a single polypeptide chain.

"Functional fragment" or "functionally equivalent fragment", as used herein, refers to a region, or fragment of a full length protein, or sequence of amino acids that, for example, comprises an active site, or any other conserved motif, relating to biological function. Functional fragments are capable of providing a biological activity substantially similar to a full-length protein disclosed herein, namely the ability to bind FasL. Functional fragments may be produced by cloning technology, or as the natural products of alternative splicing mechanisms.

"Host cell" refers to any eukaryotic or prokaryotic cell that is suitable for propagating and/or expressing a cloned gene contained on a vector that is introduced into said host cell by, for example, transformation or transfection, or the like.

"FLINT" refers to a nucleic acid and a polypeptide or amino acid sequence encoded thereby, included any FLINT polypeptide. FLINT is a member of the TNFR superfamily. This family of receptors mediates a variety of biological effects of TNF ligands, including but not limited to cell proliferation, cell differentiation, immune regulation, inflammatory response, cytotoxicity, and apoptosis.

As used herein FLINT polypeptides comprises amino acids sequences SEQ ID NOS.:2, 5,6,7,8,and 9 including but not limited to fragments thereof, especially functional fragments, analogs, homologs, orthologues, paralogues of said sequences and/or fragments which retain the biological activity of FLINT, to wit, selectively binding FasL.

The term "homolog" or "homologous" describes the relationship between different nucleic acid compounds or amino acid sequences in which said sequences or molecules are related by partial identity or similarity at one or more blocks or regions within said molecules or sequences.

The term "hybridization" as used herein refers to a process in which a single-stranded nucleic acid compound joins with a complementary strand through nucleotide base pairing. The degree of hybridization depends upon, for example, the degree of homology, the stringency of hybridization, and the length of hybridizing strands. "Selective hybridization" refers to hybridization under conditions of high stringency.

"Isolated nucleic acid compound" refers to any RNA or DNA sequence, however constructed or synthesized, which is locationally distinct from its natural location.

A "nucleic acid probe" or "probe" as used herein is a labeled nucleic acid compound that hybridizes with another nucleic acid compound. "Nucleic acid probe" means a single stranded nucleic acid sequence that will combine with a complementary or partially complementary single stranded target nucleic acid sequence to form a double-stranded molecule. A nucleic acid probe may be an oligonucleotide or a nucleotide polymer. A probe will usually contain a detectable moiety which may be attached to the end(s) of the probe or be internal to the sequence of the probe.

The term "orthologue" or "orthologous" refers to two or more genes or polypeptides from different organisms that exhibit sequence homology.

The term "paralogue" or "paralogous" refers to two or more genes or polypeptides within a single organism that exhibit sequence homology.

The term "plasmid" refers to an extrachromosomal genetic element. The plasmids disclosed herein are commercially available, publicly available on an unrestricted basis, or can be constructed from readily available plasmids in accordance with published procedures.

A "primer" is a nucleic acid fragment which functions as an initiating substrate for enzymatic or synthetic elongation of, for example, a nucleic acid compound.

The term "promoter" refers to a nucleic acid sequence that directs transcription, for example, of DNA to RNA. An inducible promoter is one that is regulatable by environmental signals, such as carbon source, heat, or metal ions, for example. A constitutive promoter generally operates at a constant level and is not regulatable.

"Recombinant DNA cloning vector" as used herein refers to any autonomously replicating agent, including, but not limited to, plasmids and phages, comprising a DNA molecule to which one or more additional DNA segments can or have been incorporated.

The term "recombinant DNA expression vector" or "expression vector" as used herein refers to any recombinant DNA cloning vector, for example a plasmid or phage, in which a promoter and other regulatory elements are present thereby enabling transcription of an inserted DNA, which may encode a polypeptide.

The term "selectively binding" refers to the ability of FLINT polypeptides to bind FasL but not TNFα.

The term "stringency" refers to hybridization conditions. High stringency conditions disfavor non-homologous basepairing. Low stringency conditions have the opposite effect. Stringency may be altered, for example, by temperature and salt concentration.

"Low stringency" conditions comprise, for example, a temperature of about 37° C or less, a formamide concentration of less than about 50%, and a moderate to low salt (SSC) concentration; or, alternatively, a temperature of about 50° C or less, and a moderate to high salt (SSPE) concentration, for example 1M NaCl.

"High stringency" conditions comprise, for example, a temperature of about 42° C or less, a formamide concentration of less than about 20%, and a low salt (SSC) concentration; or, alternatively, a temperature of about 65° C, or less, and a low salt (SSPE) concentration. For example, high stringency conditions comprise hybridization in 0.5 M $NaHPO_4$, 7% sodium dodecyl sulfate (SDS), 1 mM EDTA at 65°C (Ausubel, F.M. *et al.* Current Protocols in Molecular Biology, Vol. I, 1989; Green Inc. New York, at 2.10.3).

"SSC" comprises a hybridization and wash solution. A stock 20X SSC solution contains 3M sodium chloride, 0.3M sodium citrate, pH 7.0.

"SSPE" comprises a hybridization and wash solution. A 1X SSPE solution contains 180 mM NaCl, 9 mM Na_2HPO_4, 0.9 mM NaH_2PO_4 and 1 mM EDTA, pH 7.4.

"Substantially pure" used in reference to a peptide or protein means that said peptide or protein is separated from

other cellular and non-cellular molecules, including other protein molecules. A substantially pure preparation would be about at least 85% pure; preferably about at least 95% pure. A "substantially pure" protein as described herein could be prepared by a variety of techniques well known to the skilled artisan, including, for example, the IMAC protein purification method.

The term "vector" as used herein refers to a nucleic acid compound used for introducing exogenous or endogenous DNA into host cells. A vector comprises a nucleotide sequence that may encode one or more protein molecules. Plasmids, cosmids, viruses, and bacteriophages, in the natural state or which have undergone recombinant engineering, are examples of commonly used vectors.

The various restriction enzymes disclosed and described herein are commercially available and the manner of use of said enzymes including reaction conditions, cofactors, and other requirements for activity are well known to one of ordinary skill in the art. Reaction conditions for particular enzymes were carried out according to the manufacturer's recommendation.

Methods of Preventing or treating Disease Using FLINT

It has been discovered that FLINT polypeptides can specifically bind to Fas ligand and subsequently block Fas L-induced apoptosis. Galle et al, (J. Exp. Med., November 1995, 182:1223-1230). Galle discussed that FasL-Fas mediated cell death played a role in liver failure in humans. Galle et al knew that FasL-Fas mediated apoptosis was a mechanism to eliminate senescent hepatocytes.

The FLINT gene comprises a nucleotide sequence of 900 nucleotide base pairs (SEQ ID NO:1) that encodes a polypeptide of 300 amino acid residues in length (SEQ ID

NO:2). The FLINT gene identified from colon cells has a 87 nucleotide base pair sequence at the 5' end (i.e., nucleotides 1-87 of SEQ ID NO:1) that encodes a 29 residue signal peptide, MRALEGPGLSLLCLVLALPALLPVPAVRG (SEQ. ID NO.:11) (i.e., residues 1-29 of SEQ ID NO:2), which peptide is cleaved from the N-terminus upon secretion of the mature soluble protein (i.e., residues 30-300, SEQ ID NO:5).

Using the FLINT gene, one can produce recombinant FLINT polypeptide (as used herein the term polypeptide is intended to also include the term protein). It is possible to isolate orthologous genes from other organisms and/or paralogous genes from the same organism.

Those skilled in the art will recognize that owing to the degeneracy of the genetic code (i.e. 64 codons which encode 20 amino acids), numerous "silent" substitutions of nucleotide base pairs could be introduced into the sequence identified as SEQ ID NO:1 without altering the identity of the encoded amino acid(s) or protein product. All such substitutions are intended to be within the scope of the invention.

Also contemplated by the present invention are FLINT polypeptides and proteins and related functional fragments such as, for example, smaller alternatively spliced forms, or substitutions in which the primary sequence disclosed in SEQ ID NO:2 is altered by substitution or replacement or deletion or insertion at one or more amino acid positions, such that biological function is maintained. Functional fragments are conveniently identified as fragments of an intact FLINT polypeptide or protein that retain the capacity to bind FasL.

Several structural motifs have been identified within the primary sequence of FLINT polypeptide that are thought to be

important for biological function. For example, four cysteine rich motifs in the N-terminal domain, which are represented in a variety of related proteins, and which can form internal disulfide bonds, span from amino acid residue 34 to 195 (SEQ ID NO.: 6) of SEQ ID NO:2. Another region of the FLINT polypeptide SEQ ID NO.:2 spanning from amino acid residue 30 to 194 (SEQ. ID NO.:7), inclusive, is an important region believed to retain bioactivity. The signal peptide comprises 29 amino acids MRALEGPGLSLLCLVLALPALLPVPAVRG (SEQ. ID NO.:11) or residues 1-29 inclusive in SEQ ID NO.:2. As mentioned earlier, this signal peptide is cleaved off in the mature polypeptide. Additionally, in SEQ ID NO. 2 the region comprising residues 72-151 (SEQ ID NO.:8) is believed to retain biological function. This region can be further subdivided into subregions 1 and 2. Subregion 1 is a 43 amino acid stretch comprising residues 72-114 inclusive(SEQ ID NO.:9). Subregion 2 is a 37 amino acid stretch comprising residues 115-151 inclusive(SEQ ID NO.:10).

Functional analogs of the FLINT polypeptides are typically generated by deletion, insertion, or substitution of a single (or few) amino acid residues. Substitution modifications can generally be made in accordance with the following Table.

Table 1

ORIGINAL RESIDUE	EXEMPLARY SUBSTITUTIONS
ALA	SER
ARG	LYS
ASN	GLN, HIS
ASP	GLU
CYS	SER
GLN	ASN
GLU	ASP
GLY	PRO
HIS	ASN, GLN
ILE	LEU, VAL
LEU	ILE, VAL
LYS	ARG, GLN, GLU
MET	LEU, ILE
PHE	MET, LEU, TYR
SER	THR
THR	SER
TRP	TYR
TYR	TRP, PHE
VAL	ILE, LEU

Fragments of polypeptides

One embodiment of the instant invention provides fragments of the polypeptides disclosed that may or may not be biologically active. Such fragments are useful, for example, as an antigen for producing an antibody to said polypeptides.

Fragments of the polypeptides disclosed herein may be generated by any number of suitable techniques, including chemical synthesis of any portion of SEQ ID NO:2, proteolytic digestion of SEQ ID NO:2, or most preferably, by recombinant DNA mutagenesis techniques, well known to the

skilled artisan. *See. e.g.* K. Struhl, "Reverse biochemistry: Methods and applications for synthesizing yeast polypeptides *in vitro*," *Meth. Enzymol.* 194:520-535. For example, in a preferred method, a nested set of deletion mutations are introduced into the intact gene encoding the native FLINT polypeptide such that varying amounts of the polypeptide coding region are deleted, either from the amino terminal end, or from the carboxyl end of the polypeptide molecule. This method can also be used to create internal fragments of the intact polypeptide in which both the carboxyl and amino terminal ends are removed. Several appropriate nucleases can be used to create such deletions, for example *Bal*31, or in the case of a single stranded nucleic acid compound, mung bean nuclease. For simplicity, it is preferred that the intact FLINT gene be cloned into a single-stranded cloning vector, such as bacteriophage M13, or equivalent. If desired, the resulting gene deletion fragments can be subcloned into any suitable vector for propagation and expression of said fragments in any suitable host cell.

The present invention also provides fragments of the polypeptides disclosed herein wherein said fragments retain biological activity. As used herein, "functional fragments" includes fragments of SEQ ID NO:2 that retain and exhibit, under appropriate conditions, measurable biological activity, for example, the capacity to bind FasL.

Functional fragments of the polypeptides disclosed herein may be produced as described above, preferably using cloning techniques to engineer smaller versions of the intact gene, lacking sequence from the 5' end, the 3' end, from both ends, or from an internal site.

Gene Isolation Procedures

Those skilled in the art will recognize that the FLINT gene could be obtained by a plurality of recombinant DNA techniques including, for example, hybridization, polymerase chain reaction (PCR) amplification, or de novo DNA synthesis. (*See e.g.*, T. Maniatis *et al.* Molecular Cloning: A Laboratory Manual, 2d Ed. Chap. 14 (1989)).

Methods for constructing cDNA libraries in a suitable vector such as a plasmid or phage for propagation in prokaryotic or eukaryotic cells are well known to those skilled in the art. (*See e.g.* Maniatis *et al. Supra*). Suitable cloning vectors are well known and are widely available.

The FLINT gene or fragment thereof can be isolated from any tissue in which said gene is expressed. In one method, mRNA is isolated from a suitable tissue, and first strand cDNA synthesis is carried out. A second round of DNA synthesis can be carried out for the production of the second strand. If desired, the double-stranded cDNA can be cloned into any suitable vector, for example, a plasmid, thereby forming a cDNA library. Oligonucleotide primers targeted to any suitable region of SEQ ID NO:1 can be used for PCR amplification of FLINT. *See e.g.* PCR Protocols: A Guide to Method and Application, Ed. M. Innis et al., Academic Press (1990). The PCR amplification comprises template DNA, suitable enzymes, primers, and buffers, and is conveniently carried out in a DNA Thermal Cycler (Perkin Elmer Cetus, Norwalk, CT). A positive result is determined by detecting an appropriately-sized DNA fragment following agarose gel electrophoresis.

Polypeptide Production Methods

One embodiment of the present invention relates to the substantially purified polypeptide encoded by the FLINT gene.

Skilled artisans will recognize that the polypeptides of the present invention can be synthesized by a number of different methods, such as chemical methods well known in the art, including solid phase peptide synthesis or recombinant methods. Both methods are described in U.S. Patent 4,617,149, incorporated herein by reference.

The principles of solid phase chemical synthesis of polypeptides are well known in the art and may be found in general texts in the area. *See, e.g.*, H. Dugas and C. Penney, Bioorganic Chemistry (1981) Springer-Verlag, New York, 54-92. For example, peptides may be synthesized by solid-phase methodology utilizing an Applied Biosystems 430A peptide synthesizer (Applied Biosystems, Foster City, CA) and synthesis cycles supplied by Applied Biosystems.

The polypeptides of the present invention can also be produced by recombinant DNA methods using the cloned FLINT gene. Recombinant methods are preferred if a high yield is desired. Expression of the cloned gene can be carried out in a variety of suitable host cells, well known to those skilled in the art. For this purpose, the FLINT gene is introduced into a host cell by any suitable means, well known to those skilled in the art. While chromosomal integration of the cloned gene is within the scope of the present invention, it is preferred that the gene be cloned into a suitable extra-chromosomally maintained expression vector so that the coding region of the FLINT gene is operably-linked to a constitutive or inducible promoter.

The basic steps in the recombinant production of the FLINT polypeptide are:

a) constructing a natural, synthetic or semi-synthetic DNA encoding FLINT polypeptide;

b) integrating said DNA into an expression vector in a manner suitable for expressing the FLINT polypeptide, either alone or as a fusion polypeptide;

c) transforming or otherwise introducing said vector into an appropriate eukaryotic or prokaryotic host cell forming a recombinant host cell;

d) culturing said recombinant host cell in a manner to express the FLINT polypeptide; and

e) recovering and substantially purifying the FLINT polypeptide by any suitable means well known to those skilled in the art.

Expressing Recombinant FLINT Polypeptide in Prokaryotic and Eukaryotic Host Cells

Prokaryotes may be employed in the production of recombinant FLINT polypeptide. For example, the *Escherichia coli* K12 strain 294 (ATCC No. 31446) is particularly useful for the prokaryotic expression of foreign polypeptides. Other strains of *E. coli*, bacilli such as *Bacillus subtilis*, enterobacteriaceae such as *Salmonella typhimurium* or *Serratia marcescans*, various Pseudomonas species and other bacteria, such as *Streptomyces*, may also be employed as host cells in the cloning and expression of the recombinant polypeptides of this invention.

Promoter sequences suitable for driving the expression of genes in prokaryotes include β-lactamase [*e.g.* vector pGX2907, ATCC 39344, contains a replicon and β-lactamase gene], lactose systems [Chang et al., *Nature* (London),

275:615 (1978); Goeddel et al., *Nature* (London), 281:544 (1979)], alkaline phosphatase, and the tryptophan (trp) promoter system [vector pATH1 (ATCC 37695)], which is designed to facilitate expression of an open reading frame as a trpE fusion polypeptide under the control of the trp promoter. Hybrid promoters such as the tac promoter (isolatable from plasmid pDR540, ATCC-37282) are also suitable. Still other bacterial promoters, whose nucleotide sequences are generally known, may be ligated to DNA encoding the polypeptide of the instant invention, using linkers or adapters to supply any required restriction sites. Promoters for use in bacterial systems also will contain a Shine-Dalgarno sequence operably-linked to the DNA encoding the desired polypeptides. These examples are illustrative rather than limiting.

The polypeptides of this invention may be synthesized either by direct expression or as a fusion polypeptide comprising the polypeptide of interest as a translational fusion with another polypeptide or peptide that may be removed by enzymatic or chemical cleavage. It is often observed in the production of certain peptides in recombinant systems that expression as a fusion polypeptide prolongs the lifespan, increases the yield of the desired peptide, or provides a convenient means of purifying the polypeptide. This is particularly relevant when expressing mammalian polypeptides in prokaryotic hosts. A variety of peptidases (e.g. enterokinase and thrombin) which cleave a polypeptide at specific sites or digest the peptides from the amino or carboxy termini (*e.g.* diaminopeptidase) of the peptide chain are known. Furthermore, particular chemicals (*e.g.* cyanogen bromide) will cleave a polypeptide chain at specific sites. The skilled artisan will appreciate the

modifications necessary to the amino acid sequence (and synthetic or semi-synthetic coding sequence if recombinant means are employed) to incorporate site-specific internal cleavage sites. *See e.g.*, P. Carter, "Site Specific Proteolysis of Fusion Polypeptides", Chapter 13, in Protein Purification: From Molecular Mechanisms to Large Scale Processes, American Chemical Society, Washington, D.C. (1990).

In addition to prokaryotes, a variety of amphibian expression systems such as frog oocytes, and mammalian cell systems can be used. The choice of a particular host cell depends to some extent on the particular expression vector used. Exemplary mammalian host cells suitable for use in the present invention include HepG-2 (ATCC HB 8065), CV-1 (ATCC CCL 70), LC-MK_2 (ATCC CCL 7.1), 3T3 (ATCC CCL 92), CHO-K1 (ATCC CCL 61), HeLa (ATCC CCL 2), RPMI8226 (ATCC CCL 155), H4IIEC3 (ATCC CCL 1600), C127I (ATCC CCL 1616), HS-Sultan (ATCC CCL 1484), and BHK-21 (ATCC CCL 10), for example.

A wide variety of vectors are suitable for transforming mammalian host cells. For example, the pSV2-type vectors comprise segments of the simian virus 40 (SV40) genome required for transcription and polyadenylation. A large number of plasmid pSV2-type vectors have been constructed, such as pSV2-gpt, pSV2-neo, pSV2-dhfr, pSV2-hyg, and pSV2-β-globin, in which the SV40 promoter drives transcription of an inserted gene. These vectors are widely available from sources such as the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, Maryland, 20852, or the National Center for Agricultural Utilization Research, 1815 North University Street, Peoria, Illinois 61604-39999.

Promoters suitable for expression in mammalian cells include the SV40 late promoter, promoters from eukaryotic genes, such as, for example, the estrogen-inducible chicken ovalbumin gene, the interferon genes, the glucocorticoid-inducible tyrosine aminotransferase gene, the thymidine kinase gene promoter, and the promoters of the major early and late adenovirus genes.

Plasmid pRSVcat (ATCC 37152) comprises portions of a long terminal repeat of the Rous Sarcoma virus, a virus known to infect chickens and other host cells. This long terminal repeat contains a promoter which is suitable for use in the vectors of this invention. H. Gorman *et al.*, *Proc. Nat. Acad. Sci. (USA)*, 79, 6777 (1982). The plasmid pMSVi (NRRL B-15929) comprises the long terminal repeats of the Murine Sarcoma virus, a virus known to infect mouse and other host cells. The mouse metallothionein promoter has also been well characterized for use in eukaryotic host cells and is suitable for use in the present invention. This promoter is present in the plasmid pdBPV-MMTneo (ATCC 37224) which can serve as the starting material for the construction of other plasmids of the present invention.

Transfection of mammalian cells with vectors can be performed by a plurality of well-known processes including, but not limited to, protoplast fusion, calcium phosphate co-precipitation, electroporation and the like. *See, e.g.*, Maniatis *et al.*, *supra*.

Some viruses also make appropriate vectors. Examples include the adenoviruses, the adeno-associated viruses, the vaccinia virus, the herpes viruses, the baculoviruses, and the rous sarcoma virus, as described in U.S. Patent 4,775,624, incorporated herein by reference.

Eukaryotic microorganisms such as yeast and other fungi are also suitable host cells. The yeast *Saccharomyces cerevisiae* is the preferred eukaryotic microorganism. Other yeasts such as *Kluyveromyces lactis* and *Pichia pastoris* are also suitable. For expression in *Saccharomyces*, the plasmid YRp7 (ATCC-40053), for example, may be used. *See, e.g.*, L. Stinchcomb *et al.*, *Nature*, 282, 39 (1979); J. Kingsman *et al.*, *Gene*, 7, 141 (1979); S. Tschemper *et al.*, *Gene*, 10, 157 (1980). Plasmid YRp7 contains the TRP1 gene which provides a selectable marker for use in a trp1 auxotrophic mutant.

Purification of Recombinantly-Produced FLINT Polypeptide

An expression vector carrying the cloned FLINT gene is transformed or transfected into a suitable host cell using standard methods. Cells that contain the vector are propagated under conditions suitable for expression of the recombinant FLINT polypeptide. For example, if the recombinant gene has been placed under the control of an inducible promoter, suitable growth conditions would incorporate the appropriate inducer. The recombinantly-produced polypeptide may be purified from cellular extracts of transformed cells by any suitable means.

In a preferred process for polypeptide purification, the FLINT gene is modified at the 5' end to incorporate several histidine residues at the amino terminus of the FLINT polypeptide. This "histidine tag" enables a single-step polypeptide purification method referred to as "immobilized metal ion affinity chromatography" (IMAC), essentially as described in U.S. Patent 4,569,794, which hereby is incorporated by reference. The IMAC method enables rapid isolation of substantially pure recombinant

FLINT polypeptide starting from a crude extract of cells that express a modified recombinant polypeptide, as described above.

Other embodiments of the present invention comprise isolated nucleic acid sequences that encode SEQ ID NO:2, 5,6,7,8,9,10 or fragments thereof. As skilled artisans will recognize, the amino acid compounds of the invention can be encoded by a multitude of different nucleic acid sequences, owing to the degeneracy of the genetic code. Because these alternative nucleic acid sequences would encode the same amino acid sequences, the present invention further comprises these alternate nucleic acid sequences. Also contemplated are related nucleic acids that are at least about 75% identical to SEQ ID NO:1, or to their complementary sequence, or nucleic acids that hybridize to SEQ ID NO:1 under low stringency conditions. Such sequences may come, for example, from other related genes.

The FLINT gene (*viz.* SEQ ID NO:1) and related nucleic acid compounds that encode SEQ ID NO:2,5,6,7,8,9,10, or functional fragments thereof, may be produced by chemical synthetic methods. The synthesis of nucleic acids is well known in the art. *See, e.g.*, E.L. Brown, R. Belagaje, M.J. Ryan, and H.G. Khorana, *Methods in Enzymology*, 68:109-151 (1979). Fragments of the DNA sequence corresponding to the FLINT gene could be generated using a conventional DNA synthesizing apparatus, such as the Applied Biosystems Model 380A or 380B DNA synthesizers (Applied Biosystems, Inc., 850 Lincoln Center Drive, Foster City, CA 94404) using phosphoramidite chemistry, thereafter ligating the fragments so as to reconstitute the entire gene. Alternatively, phosphotriester chemistry may be employed to synthesize nucleic acids used in this invention (See, e.g., Gait, M.J.,

ed. Oligonucleotide Synthesis, A Practical Approach, (1984)).

In an alternative methodology, namely PCR, the DNA sequences disclosed and described herein, comprising, for example, a portion of all of SEQ ID NO.: 1 can be produced from a plurality of starting materials. For example, starting with a cDNA preparation (e.g. cDNA library) derived from tissue that expresses the FLINT gene, suitable oligonucleotide primers complementary to SEQ ID NO:1 or to any sub-region therein, are prepared as described in U.S. Patent No. 4,889,818, hereby incorporated by reference. Other suitable protocols for the PCR are disclosed in PCR Protocols: A Guide to Method and Applications, Ed. Michael A. Innis et al., Academic Press, Inc. (1990). Using PCR, any region of the FLINT gene can be targeted for amplification such that full or partial length gene sequences may be produced.

The ribonucleic acids of the present invention may be prepared using polynucleotide synthetic methods discussed *supra*, or they may be prepared enzymatically, for example, using RNA polymerase to transcribe a FLINT DNA template.

The most preferred systems for preparing the ribonucleic acids of the present invention employ the RNA polymerase from the bacteriophage T7 or the bacteriophage SP6. These RNA polymerases are highly specific, requiring the insertion of bacteriophage-specific sequences at the 5' end of the template to be transcribed. *See*, Maniatis *et al.*, *supra*.

This invention also provides nucleic acids, RNA or DNA, that are complementary to SEQ ID NO:1, or fragments thereof.

Nucleic Acid Probes

The present invention also provides probes and primers useful for a variety of molecular biology techniques including, for example, hybridization screens of genomic, subgenomic, or cDNA libraries, as well as hybridization against nucleic acids derived from cell lines or tissues that originate from drug-resistant tumors. Such hybridization screens are useful as methods to identify homologous and/or functionally related sequences from the same or other organisms, and further for investigating the mechanism by which drug resistance arises in various cancers. A nucleic acid compound comprising SEQ ID NO:1 or a complementary sequence thereof, or fragment thereof, which is at least 14 base pairs in length, and which will selectively hybridize to human DNA or mRNA encoding FLINT polypeptide or fragment thereof, or a functionally related polypeptide, is provided. Preferably, the 14 or more base pair compound is DNA. *See e.g.* B. Wallace and G. Miyada, "Oligonucleotide Probes for the Screening of Recombinant DNA Libraries," In *Meth. Enzym.*, 152, 432-442, Academic Press (1987).

Probes and primers can be prepared by enzymatic or recombinant methods, well known to those skilled in the art (*See e.g.* Sambrook et al. *supra*). A probe may be a single stranded nucleic acid sequence which is complementary in some particular degree to a nucleic acid sequence sought to be detected ("target sequence"). A probe may be labeled with a detectable moiety such as a radio-isotope, antigen, or chemiluminescent moiety. A description of the use of nucleic acid hybridization as a procedure for the detection of particular nucleic acid sequences is described in U.S. Patent No. 4,851,330 to Kohne, entitled "Method for

Detection, Identification and Quantitation of Non-Viral Organisms."

DNA sequence information provided by the present invention allows for the preparation of relatively short DNA (or RNA) sequences having the ability to specifically hybridize to gene sequences disclosed herein. In these aspects, nucleic acid probes of an appropriate length are prepared. The ability of such nucleic acid probes to specifically hybridize to a polynucleotide encoding a FLINT gene or related sequence lends particular utility in a variety of embodiments. Most importantly, the probes may be used in a variety of assays for detecting the presence of complementary sequences in a given sample.

In certain embodiments, it is advantageous to use oligonucleotide primers. The sequence of such primers is designed using a polynucleotide of the present invention for use in detecting, amplifying, or mutating a defined segment of a gene or polynucleotide that encodes a FLINT polypeptide using PCR technology.

Preferred nucleic acid sequences employed for hybridization studies, or assays, include probe molecules that are complementary to at least an about 14- to an about 70-nucleotide long stretch of a polynucleotide that encodes a FLINT polypeptide, such as the nucleotide base sequences designated as SEQ ID NO:1. A length of at least 14 nucleotides helps to ensure that the fragment is of sufficient length to form a duplex molecule that is both stable and selective. Molecules having complementary sequences over stretches greater than 14 bases in length are generally preferred in order to increase stability and selectivity of the hybrid. One will generally prefer to design nucleic acid compounds having gene-complementary

stretches of 25 to 40 nucleotides, 55 to 70 nucleotides, or even longer where desired. Such fragments may be readily prepared by, for example, directly synthesizing the fragment by chemical means, by application of nucleic acid reproduction technology, such as the PCR TM technology of U.S. Pat. No. 4,603,102, herein incorporated by reference, or by excising selected DNA fragments from recombinant plasmids containing appropriate inserts and suitable restriction enzyme sites.

The following guidelines are useful for designing probes with desirable characteristics. The extent and specificity of hybridization reactions are affected by a number of factors that determine the sensitivity and specificity of a particular probe, whether perfectly complementary to its target or not. The affect of various experimental parameters and conditions are well known to those skilled in the art.

First, the stability of the probe:target nucleic acid hybrid should be chosen to be compatible with the assay conditions. This may be accomplished by avoiding long A and T rich sequences, by terminating the hybrids with G:C base pairs and by designing a probe with an appropriate Tm (i.e. melting temperature). The melting profile, including the Tm of a hybrid comprising an oligonucleotide and target sequence, may be determined using a Hybridization Protection Assay. The probe should be chosen so that the length and percent GC content result in a Tm about 2°-10° C higher than the temperature at which the final assay will be performed. The base composition of the probe is also a significant factor because G-C base pairs exhibit greater thermal stability as compared to A-T base pairs. Thus, hybridization involving complementary nucleic acids of

higher G-C content will be more stable at higher temperatures.

The ionic strength and incubation temperature under which a probe will be used should also be taken into account. It is known that hybridization will increase as the ionic strength of the reaction mixture increases, and that the thermal stability of molecular hybrids will increase with increasing ionic strength. On the other hand, chemical reagents such as formamide, urea, DMSO and alcohols, which disrupt hydrogen bonds, increase the stringency of hybridization. Destabilization of hydrogen bonds by such reagents can greatly reduce the Tm. In general, optimal hybridization for synthetic oligonucleotide probes of about 10-50 bases in length occurs approximately 5° C below the melting temperature for a given duplex. Incubation at temperatures below the optimum may allow mismatched base sequences to hybridize and can therefore result in reduced specificity.

The length of the target nucleic acid sequence and, accordingly, the length of the probe sequence can also be important. In some cases, there may be several sequences from a particular region, varying in location and length, which will yield probes with the desired hybridization characteristics. In other cases, one sequence may be significantly better than another even though the one sequence differs merely by a single base. Finally, there can be intramolecular and intermolecular hybrids formed within a probe if there is sufficient self-complementarity. Such structures can be avoided through careful probe design. Computer programs are available to search for this type of interaction.

A probe molecule may be used for hybridizing to a sample suspected of possessing a FLINT or FLINT-related nucleotide sequence. The hybridization reaction is carried out under suitable conditions of stringency.

Alternatively, such DNA molecules may be used in a number of techniques including their use as: (1) diagnostic tools to detect polymorphisms in DNA samples from a human or other mammal; (2) means for detecting and isolating homologs of FLINT and related polypeptides from a DNA library potentially containing such sequences; (3) primers for hybridizing to related sequences for the purpose of amplifying those sequences; and (4) primers for altering the native FLINT DNA sequences; as well as other techniques which rely on the similarity of the DNA sequences to those of the FLINT DNA segments herein disclosed.

Once synthesized, oligonucleotide probes may be labeled by any of several well-known methods. *See e.g.* Maniatis *et.al.*, Molecular Cloning (2d ed. 1989). Useful labels include radioisotopes, as well as non-radioactive reporting groups. Isotopic labels include H^3, S^{35}, P^{32}, I^{125}, Cobalt, and C^{14}. Most methods of isotopic labeling involve the use of enzymes and include methods such as nick-translation, end-labeling, second strand synthesis, and reverse transcription. When using radio-labeled probes, hybridization can be detected by autoradiography, scintillation counting, or gamma counting. The detection method selected will depend upon the hybridization conditions and the particular radioisotope used for labeling.

Non-isotopic materials can also be used for labeling, and may be introduced internally into the sequence or at the end of the sequence. Modified nucleotides may be

incorporated enzymatically or chemically, and chemical modifications of the probe may be performed during or after synthesis of the probe, for example, by the use of non-nucleotide linker groups. Non-isotopic labels include fluorescent molecules, chemiluminescent molecules, enzymes, cofactors, enzyme substrates, haptens or other ligands.

In a preferred embodiment of the invention, the length of an oligonucleotide probe is greater than or equal to about 18 nucleotides and less than or equal to about 50 nucleotides. Labeling of an oligonucleotide of the present invention may be performed enzymatically using [^{32}P]-labeled ATP and the enzyme T4 polynucleotide kinase.

Vectors

Another aspect of the present invention relates to recombinant DNA cloning vectors and expression vectors comprising the nucleic acids of the present invention. The preferred nucleic acid vectors are those which comprise DNA, in particular SEQ ID NO:1, more particularly nucleotides 88-900 of SEQ ID NO:1, and more particularly nucleotides 102-585 of SEQ ID NO:1.

The skilled artisan understands that choosing the most appropriate cloning vector or expression vector depends upon a number of factors including the availability of restriction enzyme sites, the type of host cell into which the vector is to be transfected or transformed, the purpose of the transfection or transformation (e.g., stable transformation as an extrachromosomal element, or integration into the host chromosome), the presence or absence of readily assayable or selectable markers (e.g., antibiotic resistance and metabolic markers of one type and

another), and the number of copies of the gene desired in the host cell.

Vectors suitable to carry the nucleic acids of the present invention comprise RNA viruses, DNA viruses, lytic bacteriophages, lysogenic bacteriophages, stable bacteriophages, plasmids, viroids, and the like. The most preferred vectors are plasmids.

When preparing an expression vector the skilled artisan understands that there are many variables to be considered, for example, whether to use a constitutive or inducible promoter. The practitioner also understands that the amount of nucleic acid or polypeptide to be produced dictates, in part, the selection of the expression system. Regarding promoter sequences, inducible promoters are preferred because they enable high level, regulatable expression of an operably-linked gene. The skilled artisan will recognize a number of suitable promoters that respond to a variety of inducers, for example, carbon source, metal ions, and heat. Other relevant considerations regarding an expression vector include whether to include sequences for directing the localization of a recombinant polypeptide. For example, a sequence encoding a signal peptide preceding the coding region of a gene is useful for directing the extra-cellular export of a resulting polypeptide.

The present invention also provides a method for constructing a recombinant host cell capable of expressing polypeptides comprising SEQ ID NO:2, said method comprising transforming or otherwise introducing into a host cell a recombinant DNA vector that comprises an isolated DNA sequence that encodes SEQ ID NO:2. Of course, such a method also encompasses the host cells capable of expressing functional fragments of SEQ ID NO:2. The preferred host

cell is any eukaryotic cell that can accommodate high level expression of an exogenously introduced gene or polypeptide, and that will incorporate said polypeptide into its membrane structure. Vectors for expression are those which comprise SEQ ID NO:1 or a fragment thereof. Transformed host cells may be cultured under conditions well known to skilled artisans such that SEQ ID NO:2 is expressed, thereby producing a recombinant FLINT polypeptide in the recombinant host cell.

For the purpose of identifying additional compounds having utility as regulators or modifiers of FasL-mediated apoptosis or FasL-induced inflammation, it may be desirable to identify compounds that bind the FLINT polypeptide and/or modify its activity. A method for identifying such compounds comprises the steps of admixing a substantially purified preparation of a FLINT polypeptide with a test compound, and monitoring by any suitable means a binding interaction between said polypeptide and said compound.

Functional fragments of the polypeptides disclosed herein may also be identified as having activity. For this purpose, gene fragments (prepared as described elsewhere herein) are cloned into a suitable expression vector, and transformed or transfected into a suitable host cell. The culture medium of transformed or transfected host cells is then assayed for the ability to bind FasL. The level of activity in the transformed cells is compared to a negative control in which the organism is transformed by a vector without a FLINT insert and to a positive control in which the entire FLINT polypeptide is present on the transforming vector. Fragments of FLINT that impart activity to about 30% or greater of the positive control cells are regarded as biologically functional.

Skilled artisans will recognize that IC_{50} values are dependent on the selectivity of the compound tested. For example, a compound with an IC_{50} which is less than 10 nM is generally considered an excellent candidate for drug therapy. However, a compound that has a lower affinity, but is selective for a particular target, may be an even better candidate. The skilled artisan will recognize that any information regarding the binding potential, inhibitory activity, or selectivity of a particular compound is useful toward the development of pharmaceutical products.

Formulations

The FLINT polypeptide composition will be formulated and dosed in a fashion consistent with good medical practice, taking into account the clinical condition of the individual patient (especially the side effects of treatment with FLINT polypeptide alone), the site of delivery of the FLINT polypeptide composition, the method of administration, the scheduling of administration, and other factors known to practitioners. An effective amount of polypeptide results in a statistically significant modulation of the biological activity of the selected TNFR family ligand, for example, FasL or LIGHT. The biological activity for FasL includes, but is not limited to, apoptosis and/or inflammation. The biological activity for LIGHT includes, but is not limited to, cell proliferation. LIGHT is a 29kDa type II transmembrane TNF superfamily member protein produced by activated T cells. (Mauri D.M., Immunity, 8:21-30, January 1998).

Further, an effective amount may also be determined by prevention of adverse conditions and/or by amelioration of symptoms of diseases being treated. The

"effective amount" of FLINT polypeptide for purposes herein is thus determined by such considerations.

As a general proposition, the total pharmaceutically effective amount of FLINT polypeptide administered parenterally per dose will be in the range of about 1 μg/kg/day to 10 mg/kg/day of patient body weight, although, as noted above, this will be subject to therapeutic discretion. More preferably, this dose is at least 0.01 mg/kg/day. If given continuously, the FLINT polypeptide is typically administered at a dose rate of about 1 μg/kg/hour to about 50 μg/kg/hour, either by 1-4 injections per day or by continuous subcutaneous infusions, for example, using a mini-pump. An intravenous bag solution may also be employed. The length of treatment needed to observe changes and the interval following treatment for responses to occur appears to vary depending on the desired effect.

Pharmaceutical compositions containing the FLINT of the invention may be administered orally, rectally, parenterally, intracistemally, intravaginally, intraperitoneally, topically (as by powders, ointments, drops or transdermal patch), bucally, or as an oral or nasal spray. By "pharmaceutically acceptable carrier" is meant a non-toxic solid, semisolid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type. The term "parenteral" as used herein refers to modes of administration which include intravenous, intramuscular, intraperitoneal, intrasternal, subcutaneous and intraarticular injection and infusion.

The FLINT polypeptide is also suitably administered by sustained-release systems. Suitable examples of sustained-release compositions include semi-permeable polymer matrices

in the form of shaped articles, e.g., films, or microcapsules. Sustained-release matrices include polylactides (U.S. Pat. No. 3,773.919, EP 58,481), copolymers of L-glutamic acid and gamma-ethyl-L-glutamate (Sidman, U. et al., *Biopolymers 22*:547-556 (1983)), poly (2-hydroxyethyl methacrylate) (R.Langer et al., *J. Biomed. Mater. Res. 15*:167-277 (1981), and R. Langer, *Chem. Tech. 12*:98-105 (1982)), ethylene vinyl acetate (R. Langer et al., Id.) or poly-D- (-)-3-hydroxybutyric acid (EP 133,988). Sustained-release FLINT polypeptide compositions also include liposomally entrapped FLINT polypeptides. Liposomes containing FLINT polypeptides are prepared by methods known per se: DE 3,218,121; Epstein et al., Proc. Natl. Acad. Sci. (USA) 82:3688-3692 (1985); Hwang et al., Proc. Natl. Acad. Sci. (USA) 77:4030-4034 (1980); EP 52,322; EP 36,676; EP 88,046; EDP 143,949; EP 142,641; Japanese Pat. Appl. 83-118008; U.S. Pat. Nos. 4,485,045 and 4,544,545; and EP 102,324. Ordinarily, the liposomes are of the small (about 200-800 Angstroms) unilamellar type in which the lipid content is greater than about 30 mol. percent cholesterol, the selected proportion being adjusted for the optimal TNFR polypeptide therapy.

For parenteral administration, in one embodiment, the FLINT polypeptide is formulated generally by mixing it at the desired degree of purity, in a unit dosage injectable form (solution, suspension, or emulsion), with a pharmaceutically acceptable carrier, i.e., one that is non-toxic to recipients at the dosages and concentrations employed and is compatible with other ingredients of the formulation. For example, the formulation preferably does not include oxidizing agents and other compounds that are known to be deleterious to polypeptides.

Generally, the formulations are prepared by contacting the FLINT polypeptide uniformly and intimately with liquid carriers or finely divided solid carriers or both. Then, if necessary, the product is shaped into the desired formulation. Preferably the carrier is a parenteral carrier, more preferably a solution that is isotonic with the blood of the recipient. Examples of such carrier vehicles include water, saline, Ringer's solution, and dextrose solution. Non-aqueous vehicles such as fixed oils and ethyl oleate are also useful herein, as well as liposomes.

The carrier suitably contains minor amounts of additives such as substances that enhance isotonicity and chemical stability. Such materials are non-toxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, succinate, acetic acid, and other organic acids or their salts; antioxidants such as ascorbic acid; low molecular weight (less than about ten residues) polypeptides, e.g., polyarginine or tripeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids, such as glycine, glutamic acid, aspartic acid, or arginine; monosaccharides, disaccharides, and other carbohydrates including cellulose or its derivatives, glucose, manose, or dextrins; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; counterions such as sodium; and/or nonionic surfactants such as polysorbates, poloxamers, or PEG.

The FLINT polypeptide is typically formulated in such vehicles at a concentration of about 0.1 mg/ml to 100 mg/ml, preferably 1-10 mg/ml, at a pH of about 3 to 8. It will be understood that the use of certain of the foregoing

excipients, carriers, or stabilizers will result in the formation of FLINT polypeptide salts.

FLINT polypeptides to be used for therapeutic administration must be sterile. Sterility is readily accomplished by filtration through sterile filtration membranes (e.g., 0.2 micron membranes). Therapeutic FLINT polypeptide compositions generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

FLINT polypeptides ordinarily will be stored in unit or multi-dose containers, for example, sealed ampoules or vials, as an aqueous solution or as a lyophilized formulation for reconstitution. As an example of a lyophilized formulation, 10-ml vials are filled with 5 ml of sterile-filtered 1% (w/v) aqueous FLINT polypeptide solution, and the resulting mixture is lyophilized. The infusion solution is prepared by reconstituting the lyophilized FLINT polypeptide using bacteriostatic Water-for-Injection.

The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration. In addition, the polypeptides of the present invention may be employed in conjunction with other therapeutic compounds.

Methods of Testing FLINT activity

As mentioned above, it has been discovered that FLINT polypeptides can specifically bind to Fas ligand. This binding is involved in both FasL-induced apoptosis as well as FasL induction of the processing and secretion of interleukin-1β (IL-1β) inducing massive neutrophil infiltration. Thus, FLINT is useful for the treatment of all conditions where blocking the FasL-Fas interaction is desirable. For example, FLINT blocks FasL-induced apoptosis. In apoptosis, it is believed that Fas ligand binds to its receptor, Fas, and trimerizes the receptor that subsequently binds to FADD and Caspase 8. Finally, caspases are activated and cells undergo to apoptosis. In particular, activated T cells express Fas ligand and induce apoptosis, a mechanism called activation induced cell death (AICD). Jurkat cells, upon activation *in vitro* using anti-CD3 Ab, express FasL and undergo apoptosis. This activation induced cell death is inhibited by functional blocking anti-FasL Ab or caspase inhibitor (DEVE-fmk) as discussed in the Examples. Interestingly, the cell death mediated by FasL is also strongly inhibited by FLINT but not some other TNF-superfamily members. For example, the specific inhibition by FLINT is also shown by the soluble recombinant FasL induced Jurkat apoptosis not by OPG2 and OPG1 (data not shown). Also, FLINT does not inhibit TRAIL- or TNFα-induced cell death further demonstrating that it does not inhibit all type of TNF-related apoptosis inducing ligands. This is particularly important because the use of FLINT will not affect the circulating levels of TNFα. Using FLINT polypeptides avoids problems that face anti-TNFα or soluble TNFα receptor in treatment of diseases where cytokine induced apoptosis plays an important role. Without

intending to limit the invention, it is believed that the FasL-Fas signaling is turned on as a disease progresses. FasL-Fas signaling is at its peak in the later stages of disease. TNFα is believed to be one of the first signals activated. Thus, blocking FasL-Fas signal has at least two advantages over blocking the TNFα pathway. First, the TNFα signal is not disturbed thereby protecting the beneficial role of boosting general immunity. Second, FLINT polypeptides could be used in both the early and later stages of diseases. Often in an acute disease, patients are likely to present for treatment at advanced stages of disease. This is particularly important in ARDS correlated to sepsis.

Still further, as the Examples indicate, it has been discovered that in dose dependent inhibition analysis the FLINT can inhibit anti-CD3 activation induced cell death at lowest concentration of 500 ng/ml. This evidence demonstrated that FLINT is a specific inhibitor for Fas L-mediated cells death through binding to Fas ligand.

This effect of this binding in apoptosis can be measured several ways. For example, in an in vivo model, reduced death in a treatment group is indicative of an agent's protective effect against acute inflammatory and apoptotic challenges. DNA fragmentation, indicative of apoptosis, can be quantitated using tissues obtained from treated animals according to conventional methods. A DNA ladder observed upon electrophoresis is a well-established marker of apoptosis. Other methods of identifying whether apoptosis has occurred include observing changes in cell shape (e.g., cell shrinkage), nuclear condensation and reduced mitochondrial function (e.g., cell viability in MTT assay.)

In a mouse model of acute hepatitis, administration of anti-FasL antibody to mice caused liver failure induced by apoptosis in hepatocytes and the animals die within hours. Kondo et al, 1997 Nature Medicine 3(4):409-413. See also Galle et al, J. Exp. Med, November 1995, 182:1223-1230.

The following examples more fully describe the present invention. Those skilled in the art will recognize that the particular reagents, equipment, and procedures described are merely illustrative and are not intended to limit the present invention in any manner.

EXAMPLE 1

RT-PCR Amplification of FLINT Gene from mRNA

A FLINT gene is isolated by reverse transcriptase PCR (RT-PCR) using conventional methods. Total RNA from a tissue that expresses the FLINT gene, for example, lung, is prepared using standard methods. First strand FLINT cDNA synthesis is achieved using a commercially available kit (SuperScript™ System; Life Technologies) by PCR in conjunction with specific primers directed at any suitable region of SEQ ID NO:1.

Amplification is carried out by adding to the first strand cDNA (dried under vacuum): 8 µl of 10X synthesis buffer (200 mM Tris-HCl, pH 8.4; 500 mM KCl, 25 mM $MgCl_2$, 1 ug/ul BSA); 68 µl distilled water; 1 µl each of a 10 uM solution of each primer; and 1 µl Taq DNA polymerase (2 to 5 U/µl). The reaction is heated at 94° C for 5 minutes to denature the RNA/cDNA hybrid. Then, 15 to 30 cycles of PCR amplification are performed using any suitable thermal cycle apparatus. The amplified sample may be analyzed by agarose gel electrophoresis to check for an appropriately-sized fragment.

EXAMPLE 2

Production of a Vector for Expressing FLINT in a Host Cell

An expression vector suitable for expressing FLINT or fragment thereof in a variety of prokaryotic host cells, such as *E. coli* is easily made. The vector contains an origin of replication (Ori), an ampicillin resistance gene (Amp) useful for selecting cells which have incorporated the vector following a transformation procedure, and further comprises the T7 promoter and T7 terminator sequences in operable linkage to a FLINT coding region. Plasmid pET11A (obtained from Novogen, Madison WI) is a suitable parent plasmid. pET11A is linearized by restriction with endonucleases NdeI and BamHI. Linearized pET11A is ligated to a DNA fragment bearing NdeI and BamHI sticky ends and comprising the coding region of the FLINT gene as disclosed by SEQ ID NO:1 or a fragment thereof.

The FLINT gene used in this construction may be slightly modified at the 5' end (amino terminus of encoded polypeptide) in order to simplify purification of the encoded polypeptide product. For this purpose, an oligonucleotide encoding 8 histidine residues is inserted after the ATG start codon. Placement of the histidine residues at the amino terminus of the encoded polypeptide serves to enable the IMAC one-step polypeptide purification procedure.

EXAMPLE 3

Recombinant Expression and Purification of FLINT Polypeptide

An expression vector that carries an open reading frame (ORF) encoding FLINT or fragment thereof and which ORF is

operably-linked to an expression promoter is transformed into *E. coli* BL21 (DE3)(*hsd*S *gal* λcIts857 *ind*1*Sam*7*nin*5*lac*UV5-T7gene 1) using standard methods. Transformants, selected for resistance to ampicillin, are chosen at random and tested for the presence of the vector by agarose gel electrophoresis using quick plasmid preparations. Colonies which contain the vector are grown in L broth and the polypeptide product encoded by the vector-borne ORF is purified by immobilized metal ion affinity chromatography (IMAC), essentially as described in US Patent 4,569,794.

Briefly, the IMAC column is prepared as follows. A metal-free chelating resin (e.g. Sepharose 6B IDA, Pharmacia) is washed in distilled water to remove preservative substances and infused with a suitable metal ion [e.g. Ni(II), Co(II), or Cu(II)] by adding a 50mM metal chloride or metal sulfate aqueous solution until about 75% of the interstitial spaces of the resin are saturated with colored metal ion. The column is then ready to receive a crude cellular extract containing the recombinant polypeptide product.

After removing unbound polypeptides and other materials by washing the column with any suitable buffer, pH 7.5, the bound polypeptide is eluted in any suitable buffer at pH 4.3, or preferably with an imidizole-containing buffer at pH 7.5.

EXAMPLE 4

Tissue Distribution of FLINT mRNA

The presence of FLINT mRNA in a variety of human tissues was analyzed by Northern analysis. Total RNA from different tissues or cultured cells was isolated by a

standard guanidine chloride/phenol extraction method, and poly-A^+ RNA was isolated using oligo(dT)-cellulose type 7 (Pharmacia). Electrophoresis of RNA samples was carried out in formaldehyde followed by capillary transfer to Zeta-Probe™ nylon membranes (Bio-Rad, Hercules, Calif.). SEQ ID NO:1 was the template for generating probes using a MultiPrime™ random priming kit (Amersham, Arlington Heights, Ill.). The efficiency of the labeling reaction was approximately 4 x 10^{10} cpm incorporated per µg of template. The hybridization buffer contained 0.5M sodium phosphate, 7% SDS (wt/vol), 1% BSA (wt/vol), and 1 mM EDTA. Prehybridization was carried out in hybridization buffer at 65° C for 2 h and ^{32}P-labeled probe was added and incubation continued overnight. The filters were washed in Buffer A (40 mM sodium phosphate pH 7.2, 5% SDS [wt/vol], 0.5% BSA [wt/vol], and 1 mM EDTA) at 65° C for 1 h, and then in Buffer B (40 mM sodium phosphate, pH 7.2, 1% SDS [wt/vol], and 1 mM EDTA) at 65° C for 20 minutes. The filters were air-dried and exposed to Kodak X-OMAT AR film at -80° C with an intensifying screen.

The results showed that FLINT mRNA was present in numerous tissues, including stomach, spinal cord, lymph node, trachea, spleen, and lung.

EXAMPLE 5

Production of an Antibody to a Polypeptide

Substantially pure polypeptide or fragment thereof is isolated from transfected or transformed cells using any of the well known methods in the art, or by a method specifically disclosed herein. Concentration of polypeptide in a final preparation is adjusted, for example, by filtration through an Amicon filter device such that the

level is about 1 to 5 ug/ml. Monoclonal or polyclonal antibody can be prepared as follows.

Monoclonal antibody can be prepared from murine hybridomas according to the method of Kohler and Milstein (*Nature*, 256, 495, 1975), or a modified method thereof. Briefly, a mouse is repetitively inoculated with a few micrograms of the polypeptide or fragment thereof, or fusion peptide thereof, over a period of a few weeks. The mouse is then sacrificed and the antibody producing cells of the spleen isolated. The spleen cells are fused by means of polyethylene glycol with mouse myeloma cells. Fused cells that produce antibody are identified by any suitable immunoassay, for example, ELISA, as described in E. Engvall, *Meth. Enzymol.*, 70, 419, 1980.

Polyclonal antiserum can be prepared by well known methods (*See e.g.* J. Vaitukaitis *et.al. Clin. Endocrinol. Metab.* 33, 988, 1971) that involve immunizing suitable animals with the polypeptides, fragments thereof, or fusion polypeptides thereof, disclosed herein. A small dose (e.g. nanogram amounts) of antigen administered at multiple intradermal sites appears to be the most reliable method.

EXAMPLE 6

Construction of FLINT-Flag Expression Vector

To facilitate confirmation of FLINT expression (without the use of antibodies), a bicistronic expression vector (pIG1-FLINTF) was constructed by insertion of an "internal ribosome entry site"/enhanced green fluorescent polypeptide (IRES/eGFP) PCR fragment into the mammalian expression vector pGTD (Gerlitz, B. et al., 1993, Biochemical Journal 295:131). This new vector, designated pIG1, contains the following sequence landmarks: the E1a-responsive GBMT

promoter (D. T. Berg et al., 1993 BioTechniques 14:972; D.T. Berg et al., 1992 Nucleic Acids Research 20:5485); a unique *BclI* cDNA cloning site; the IRES sequence from encephalomyocarditis virus (EMCV); the eGFP (Clontech) coding sequence (Cormack, et al., 1996 Gene 173:33); the SV40 small "t" antigen splice site/poly-adenylation sequences; the SV40 early promoter and origin of replication; the murine dihydrofolate reductase (dhfr) coding sequence; and the pBR322 ampicillin resistance marker/origin of replication.

Based upon the human FLINT sequence, the following primers were synthesized: 5'- TAGGGCTGATCAAGGATGG GCTTCTGGACTTGGGCGGCCCCTCCGCAGGCGGACCGGGG-3' (SEQ ID NO:3); and 5'- AGGGGGGCGGCCGCTGATCATCACTTGTCGTCGTCGTCCTTGTAGTCGTGCA CAGGGAGGAAGCGC - 3' (SEQ ID NO:4). The reverse primer contained the Flag epitope sequence (nucleotides 24-47 of SEQ ID NO:4) (Micele, R.M. et al., 1994 J. Immunol. Methods 167:279). These primers were then used to PCR amplify the FLINT cDNA. The resultant 1.3 Kb PCR product was then digested with *BclI* (restriction sites incorporated into primers, underlined above) and ligated into the unique *BclI* site of pIG1 to generate the plasmid pIG1-FLINTF. Restriction digest and double stranded sequencing of the insert confirmed the human FLINT cDNA orientation and nucleotide sequence.

EXAMPLE 7

Construction of FLINT-non-Flag Expression Vector

In order to generate a non-Flagged expression vector (pIG1-FLINT), the 24-base DNA sequence encoding the eight amino acid FLAG epitope was deleted from the pIG1-FLINTF construct using the Quik Change mutagenesis kit

(Stratagene). A 35-base primer, and its complement, with identity to the 19-base sequences flanking the FLAG sequence was synthesized and used to prime PCR using the plasmid as template. The PCR product was digested with *DpnI* restriction endonuclease to eliminate the parental DNA, and the digested product was transformed into Epicurean XLI-blue E.coli cells. Sixteen ampicillin-resistant transformants were picked and the plasmid DNA was analyzed by restriction digestion. Ten of the 16 gave results compatible with deletion of the 24-base sequence. Precise deletion of the 24-base sequence was confirmed by DNA sequencing of pIG1-FLINT.

EXAMPLE 8

Isolation of a high-producing FLINT clone from AV12 RGT18 transfectants

The recombinant plasmid carrying the FLINT gene encodes resistance to methotrexate. In addition, the construct contains a gene encoding a fluorescent polypeptide, GFP, on the same transcript and immediately 3' to the FLINT gene. Since high level expression of GFP would require a high level of expression of the FLINT-GFP mRNA, highly fluorescent clones would have a greater probability of producing high levels of FLINT. pIG1-FLINT and pIG1-FLINTF were used to transfect AV12 RGT18 cells. Cells resistant to 250 nM methotrexate were selected and pooled. The pool of resistant clones was subjected to fluorescence assisted cell sorting (FACS), and cells having fluorescence values in the top 5% of the population were sorted into a pool and as single cells. The high fluorescence pools were subjected to three successive sorting cycles. Pools and individual clones from the second and third cycles were analyzed for

FLINT production by SDS-PAGE. Pools or clones expressing FLINT at the highest level judged from Coomassie staining were used for scale-up and FLINT purification.

EXAMPLE 9

Large Scale FLINT Polypeptide Purification

Large scale production of FLINT was done by first growing the stable clones in several 10 liter spinners. After reaching confluency, cells were further incubated for 2-3 more days to secrete maximum amount of FLINT into media. Media containing FLINT was adjusted to 0.1% CHAPS and concentrated in an Amicon ProFlux M12 tangential filtration system to 350 ml. The concentrated media was centrifuged at 19,000 rpm (43,000 x g) for 15 minutes and passed over a SP-5PW TSK-GEL column (21.5 mm x 15 cm; TosoHaas) at a flow rate of 8 ml/min. The column was washed with buffer A(20 mM MOPS, 0.1% CHAPS, pH 6.5) until the absorbency (280 nm) returned to baseline and the bound polypeptides were eluted with a linear gradient from 0.1 M-0.3 M NaCl(in buffer A) developed over 85 min. Fractions containing FLINT were pooled and passed over a (7.5 mm x 7.5 cm) Heparin-5PW TSK-GEL column equilibrated in buffer B (50 mM Tris, 0.1% CHAPS, 0.3 M NaCl, pH 7.0). The bound polypeptide was eluted with a linear gradient from 0.3 M-1.0 M NaCl (in buffer B) developed over 60 min. Fractions containing FLINT were pooled and passed over a 1 cm x 15 cm Vydac C4 column equilibrated with 0.1% TFA/H_2O. The bound FLINT was eluted with a linear gradient from 0-100% CH_3CN/0.1% TFA. Fractions containing FLINT were analyzed by SDS-PAGE and found to be greater than 95% pure and were dialyzed against 8 mM $NaPO_4$, 0.5 M NaCl, 10% glycerol, pH 7.4. The N-terminal sequence of FLINT was confirmed on the purified

polypeptide. Mass spectral analysis and Endogylcosidase-F digestion indicates that FLINT is glycosylated.

EXAMPLE 10

FAS LIGAND BINDING EXPERIMENTS

To detect FLINT interaction with FasL.

Dot blot experiment was performed to scan known TNF ligands that are commercially available TRAIL and FasL for interaction with FLINT.

TRAIL (RnD Systems) and FasL (Kamiya Biomedical Company) were spotted on a nitrocellulose paper and incubated with purified FLINT-Flag. FLINT was washed away and binding FLINT was detected using anti Flag antibody

Both OPG2Fc and FLINT-Flag were overexpressed and purified according the examples above. The filter paper was subsequently blocked for 30 min using 5% nonfat milk in PBS in room temperature.

The nitrocellulose paper was subsequently mixed with the cell lysate containing FasL-Myc, and further incubated on a rotator for 1 hour at room temperature. Secondary and tertiary incubations were performed with anti-myc antibody and anti-mouse IgG-HRP for 1 hour and 30 minutes respectively. The polypeptide containing myc epitope was detected by chemiluminescence on X-ray film that showed that FLINT bound to FasL specifically.

First a baseline experiment was done for the Fas-FasLigand interaction *in vitro*. Unless otherwise indicated, all washing steps use TBST (Tris Buffer Saline with Tween 20 from SIGMA) and were done 3 to 6 times.

mrecFas (100 ng) was adsorbed on to ELISA plate. Then the plate was is blocked by TBST plus 0.1%Gelatine. Thereafter, hFasLigand (Flag-tagged) was added at different

concentrations with a maximum concentration of 300 ng going down to 1 ng on TBST plus a 0.1% solution containing 1 micrograms/ml of M2 Abs (antiflag antibodies purchased through Scientific Imaging System division of Kodak). After washing the plate 6 times, anti-mouse-Abs-HRP (3000 dilution, Bio-Rad) was added to the wells. After washings three times, visualization enzymatic reaction using ABTS as a substrate was performed. Unless otherwise noted, an ELISA reader (Molecular Devices, Corp., Menlo Park, California) was used.

The following data was collected:

FasL, ng	OD, 405nM
1	.1
5	.2
10	.3
50	.7
100	1.2
500	1.6

FLINT prevents Fas-FasLigand interaction

As above, mrecFas (100 ng) was adsorbed on to ELISA plate. Again the plate was blocked by TBST and 0.1%Gelatine. Thereafter, hFasLigand (Flag-tagged, 30 ng per each point) in the presence of different FLINT concentrations (Maximum concentration 300 ng down to 1 ng) on TBST plus a 0.1% solution containing 1 microgram/ml of M2 Abs is added to each well. As before, after washing of the plate, anti-mouse-Abs-HRP (3000 dilution, Bio-Rad) was added to the wells. After washings, visualization enzymatic reaction using ABTS as a substrate was performed. The data is shown in the following table.

FLINT, ng	OD, 405nM
1	0.36
5	0.36
10	0.36
50	0.28
100	0.18
500	0.06

FasLigand binds Fas and FLINT with different affinities.

FLINT and Fas (100 ng of each) were adsorbed on to an ELISA plate. hFasLigand (Flag-tagged) was added at different concentrations to a maximum concentration of 300 ng down to 0.1 ng on TBST plus a 0.1% solution containing 1 microgram/ml of M2 Abs. After washing of the plate, anti-mouse-Abs-HRP (1:3000 dilution, Bio-Rad) was added to the wells. After washings, visualization enzymatic reaction using ABTS as a substrate was performed. The table below shows the data.

FasL, ng	FLINT OD	Fas OD, 405nM
0.1	0	0
0.5	0	0
1.0	.02	0
5.0	.04	.01
10	.12	.03
50	.28	.045
100	.78	.18

EXAMPLE 11

Measuring the effect of FLINT on anti-CD3 induced Jurkat apoptosis

Non-tissue treated 24 well plates (Decton Dickinson, Mansfield, MA) were coated with 0.5 ml of 1 ug/ml anti-CD3 (Farmingen) in PBS for 90 min at 37 °C. The plate was washed once with PBS. 1 ml of 1 X 10^6 cell/ml was seated in

each well with or without following treatment: 10 μM DEVD-cmk, 1 ug OPG2-Fc, 1 or 2 ug of FLINT and 1 ug anti FasL Ab.

Cells were incubated overnight at 37 °C incubator and were then stained by Annexin V and PI staining. Apoptosis was analyzed by flow cytometer (FACS). Cell apoptosis was indicated by positive staining with Annexin V.

Control Jurkat	6.97
Jurkat + anti Fas	59.28
Jurkat + antiCD3	46.32
Jurkat + antiCD3 + DEVDcmk	30.80
Jurkat + antiCD3 + FLINT (1ug)	27.77
Jurkat + antiCD3 + OPG2-Fc (1ug)	45.78
Jurkat + antiCD3 + FLINT (2ug)	18.67
Jurkat + antiCD3 + antiFasL Ab	24.05

EXAMPLE 12

Measuring the effect of FLINT on recombinant FasL induced Jurkat cells apoptosis

1 ml of 1 X 10^6 cell/ml was added into each well of 24 well tissue culture plate and treated with following reagents: soluble Fas L (200 ng), Fas L plus 1 ug FLINT, Fas L plus 1 ug OPG2-Fc, Trail (200 ng), Trail plus 1 ug FLINT. Cells were incubated overnight at 37 °C and then stained with Annexin V and PI. Cell apoptosis was analyzed by flow cytometer (FACS).

Control Jurkat	3.23
Jurkat + FasL (200ng/ml)	67.39
Jurkat + FasL (200ng/ml) + anti FasL Ab (1 ug)	3.3
Jurkat + FasL (200ng/ml) + FLINT (1 ug)	3.32
Jurkat + FasL (200ng/ml) + FLINT (1 ug)	4.6
Jurkat + FasL (200ng/ml) + OPG2(1ug)	70.58
Jurkat + FasL (200ng/ml) + OPG2(1ug)	69.58
Jurkat + TRAIL (200ng/ml)	17.47
Jurkat + TRAIL (200ng/ml)	17.43

EXAMPLE 13

Measuring the effect of FLINT in a dose-dependent manner on anti-CD3 induced Jurkat apoptosis

The same steps for plate coating and cell treatment set out in Example 13 were followed except a different amount of FLINT was added into each well. The following table indicates the amounts added:

Jurkat cells (Control)	5.33
Jurkat cells + anti CD3	27.49
Jurkat cells + anti CD3 + anti FasL neutralization Ab	12.74
Jurkat cells + anti CD3 + OPG2-Fc 4ug	26.24
Jurkat cells + anti CD3 + FLINT/PG3 3000ng	14.68
Jurkat cells + anti CD3 + FLINT 2000ng	17.02
Jurkat cells + anti CD3 + FLINT 1000ng	24.29
Jurkat cells + anti CD3 + FLINT 500ng	27.48
Jurkat cells + anti CD3 + FLINT 250ng	28.93
Jurkat cells + anti CD3 + FLINT 125ng	29.4

Jurkat cells + anti CD3 + FLINT 62.5ng	28.99
Jurkat cells + anti CD3 + FLINT 31.25ng	28.21
Jurkat cells + anti CD3 + FLINT 15.625ng	28.80

EXAMPLE 14

Measuring the effect of human FLINT on murine Fasl-mediated apoptosis using mouse T cell hybridoma cells (LTT cells (Annexin V assay)

LTT,2,14,11 cells (LTT cells) (Glasebrook, A.L., 1987 Eur.J.Immunol, 17:1561-1565 were used in this Annexin V assay. On the first day, a 96 well plate was coated with anti-CD3 (2C11) at a serial dilutions. On the second day, 100,000 LTT cells were added(in 50ul of medium) per well along with 50 ul of medium to the control wells and 50 ul of medium containing :

Group 1. soluble Fas (sFas)(FasFc, mouse), with a final concentration of 1 ug/ml

Group 2. FLINT (human), with a final concentration of 1 ug/ml

Group 3. anti-FasL (mouse), with a final concentration of 1 ug/ml.

These were then incubated at 37° C, 5% CO_2, overnight.

On the following day (Day3). The cells were collected from each well, washed and labeled with Annexin V and PI, following Flow cytometry analysis.

Anti CD3	Control % Annexin V Positive	sFas % Annexin V Positive	FLINT % Annexin V Positive	Anti-FasL % Annexin V Positive
1	96.42	56.66	34.41	53.46
0.33	94.48	47.28	35.89	39.21
0.11	91.27	41.08	33.24	32.54
0	19.74	23.14	26.47	17.54

EXAMPLE 15

Measuring the effect of human FLINT on murine Fasl-mediated apoptosis using mouse T cell hybridoma cells (LTT cells (Cytotoxicity Assay)

The same steps as in Example 16 were followed on Day 1 and Day 2. On Day 3 20ul of MTS solution (Promega) was added to the cells which were then incubated at 37°C for 2 hours. Using a plate reader, the absorbences at 490nm wavelength were collected.

Anti-CD3 conc. ug/ml)	s-Fas (1ug/ml	FLINT 1ug/ml)	Anti-FasL	Control
0	1.774	1.691	2.01	1.534
0.0014	1.968	1.923	2.134	1.614
0.004	1.929	1.982	2.147	1.653
0.012	1.779	2.006	2.108	1.284
0.037	1.777	2.006	1.988	0.834
0.11	1.638	1.874	1.956	0.733
0.33	1.624	1.671	1.978	0.648
1	1.459	1.581	1.887	0.664

EXAMPLE 16

LIGHT Binding experiments

To confirm the dot blot binding between LIGHT and FLINT, 293 cells were transiently transfected with LIGHT expression construct overnight. Next day cells were detached and incubated with FLINT-Flag on ice. The Flag epitope was subsequently detected by anti-Flag conjugated with fluorochrome and the cells population that shows specific binding with FLINT was detected by flow cytometer. As a control we used vector transfected cells. To make sure that the binding was specific, a competition assay with 10 fold access of untagged FLINT was performed.

More particularly, 6 well dishes of cells transfected as above. Both vector and m-LIGHT expressing cells were provided. Cells were detached from the plates by vigorous pipetting with a P100 Pipettor. Then, in PBS/BSA, 0.1%, cells were exposed to either:

a. GST/flag, FLINT/flag, or HVEM at 20 nM or;

b. FLINT/flag at 20 nM + either GST/flag, FLINT, HVEM at 200 nM or;

c. FLINT/flag at 20 nM + HVEM + anti-human FAS ligand at 200 nM;

d. anti-human FAS ligand-biotin at 1 µg/ml;

Cells were incubated on ice for 30 minutes and washed with PBS/BSA, 0.1%. Cells were then exposed to either anti-human IgG-biotin, 1µg/ml (for detection of HVEM), M2-biotin, 2 µg/ml (for detection of flag conjugates). Cells were incubated on ice for 30 minutes and washed with PBS/BSA, 0.1%. Thereafter, cells were exposed to streptavidin Alexa 488 (SIGMA) at a 1:1000 dilution. Again, cells were

incubated on ice for 30 minutes and washed with PBS/BSA, 0.1%. Cells were analyzed using a FACSORT flow cytometer (Decton Dickinson) to determine binding.

The cell surface binding assay using flow cytometer confirmed that peaks shifted only when LIGHT expressing cells were stained with FLINT-Flag was used (data not shown). There was no shift in the control cells when stained with FLINT-Flag. The shifted peak was completely reversed to baseline peak when the cells were preincubated with 10 fold excess of non tagged FLINT thereby preoccupying all the binding sites for FLINT-Flag.

EXAMPLE 17

In vivo testing of FLINT for treatment of Liver Damage

Using a mouse or rat, a model of liver damage was induced using the methods set out in Tsuji H., et al, 1997, Infection and Immunity, 65(5):1892-1898. After priming with heat killed bacteria such as *Propionibacterium acnes*, challenge with a low dose of lipopolysaccaride (LPS) induced acute and massive hepatic injury. Specifically, the activity of the polypeptides of the present invention against acute inflammation and apoptosis is determined using a modification of the procedure reported by Tsuji et al., supra. Briefly, BALB/c mice (Harlan) per each experimental group are given intravenous injections (the lateral tail vein) of 6 mg of D(+)-Galactosamine (Sigma, 39F-0539) in 100 µl of PBS (GIBCO-BRL) and 3 µg of Lipopolysaccharide B *E.coli* 026:B6 (LPS) (Difco, 3920-25-2) in 100 µl of PBS. After LPS challenge, the animals were injected intraperitoneally with FLINT (200 µg), Hamster IgG (500 µg, Cappel, 30926), mAb against murine TNF, TN3-19.12 (500 µg,

Sheehan K.C.F. et al J. Immunol. 1989. 142: 3884), and Anti-mouse Fas Ligand (500 µg, PharMingen, MO24301) at 0, 2, 4, 6 hour-point respectively. The survival rates of the mice were determined 24 and 48 hours after LPS injection.

In mice challenged with 3 µg of LPS after IP injection of 200 µg FLINT polypeptide (FLINT) had a positive effect on animal survival. When FLINT was administered 2 hours post-challenge, 100% of the animals survived; at 4 hours post- challenge, 73% of the animals survived; at 6 hours post-challenge, 60% of the animals survived. In contrast, administration of anti-TNFα at 4 hours post-challenge protected only 10% of the animals.

EXAMPLE 18

In vivo testing of FLINT for treatment of Septic Shock

To test the action of FLINT in a septic shock model, a modification of the procedure in Example 17 was followed. The animals were given with 200 µg of FLINT at various points in time relative to challenge with 200µg of LPS. Without treatment, it is expected that such a dose of LPS would be lethal to the mice. The results showed that pretreatment of animals with 200 µg of FLINT protected 80% of animals from lethality induced with 200µg of LPS; co-administration with LPS challenge resulted in 60% protection; while 2 hour post-challenge administration did not have protective effect. Thus, FLINT demonstrates protection of mice in a septic shock model.

EXAMPLE 19

In vivo testing of FLINT for treatment or prevention of ARDS

Rabbits are exposed to hyperoxia (100% O_2) for 64 hours develop clinical symptoms that have been recognized as very similar to human ARDS. For example one molecular endpoint of the hyperoxia model is increased alveolar permeability to solute, which can be quantitated. Rabbits are challenged with hyperoxia to induce the ARDS symptomology and then treated with FLINT polypeptides and solute permeability across the alveolar epithelium is measured.

The polypeptides of varying concentrations are given at different times to determine it usefulness as a prophylactic (before challenge) and/or treatment (before, during and/or after challenge).

FLINT polypeptides are expected to improve lung function. One measurement is fluid transport across the alveoli.

SEQUENCE LISTING

<110> Dou, Shenshen
Glasebrook, Andrew L.
Na, Songqing
Noblitt, Timothy W.
Song, Ho Yeong

<120> Method of Treating Acute Respiratory Distress Syndrome (ARDS) Using FLINT Polypeptides

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             20                  25                  30

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         35                  40                  45
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 65                  70                  75                  80

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         35                  40                  45

Cys Ala Gln Cys Pro Pro Gly Thr Phe Val Gln Arg Pro Cys Arg Arg
     50                  55                  60

Asp Ser Pro Thr Thr Cys Gly Pro Cys Pro Pro Arg His Tyr Thr Gln
 65                  70                  75                  80

Phe Trp Asn Tyr Leu Glu Arg Cys Arg Tyr Cys Asn Val Leu Cys Gly
                 85                  90                  95

Glu Arg Glu Glu Glu Ala Arg Ala Cys His Ala Thr His Asn Arg Ala
            100                 105                 110

Cys Arg Cys Arg Thr Gly Phe Phe Ala His Ala Gly Phe Cys Leu Glu
        115                 120                 125

His Ala Ser Cys Pro Pro Gly Ala Gly Val Ile Ala Pro Gly Thr Pro
    130                 135                 140

Ser Gln Asn Thr Gln Cys Gln Pro Cys Pro Pro Gly Thr Phe Ser Ala
145                 150                 155                 160

Ser Ser Ser Ser Ser Glu Gln Cys Gln Pro His Arg Asn Cys Thr Ala
                165                 170                 175

Leu Gly Leu Ala Leu Asn Val Pro Gly Ser Ser Ser His Asp Thr Leu
            180                 185                 190

Cys Thr Ser Cys Thr Gly Phe Pro Leu Ser Thr Arg Val Pro Gly Ala
        195                 200                 205

Glu Glu Cys Glu Arg Ala Val Ile Asp Phe Val Ala Phe Gln Asp Ile
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Cys Arg Arg Asp Ser Pro Thr Thr Cys Gly Pro Cys Pro Pro Arg His
         35                  40                  45

Tyr Thr Gln Phe Trp Asn Tyr Leu Glu Arg Cys Arg Tyr Cys Asn Val
     50                  55                  60

Leu Cys Gly Glu Arg Glu Glu Glu Ala Arg Ala Cys His Ala Thr His
 65                  70                  75                  80

Asn Arg Ala Cys Arg Cys Arg Thr Gly Phe Phe Ala His Ala Gly Phe
                 85                  90                  95

Cys Leu Glu His Ala Ser Cys Pro Pro Gly Ala Gly Val Ile Ala Pro
            100                 105                 110

Gly Thr Pro Ser Gln Asn Thr Gln Cys Gln Pro Cys Pro Pro Gly Thr
        115                 120                 125

Phe Ser Ala Ser Ser Ser Ser Ser Glu Gln Cys Gln Pro His Arg Asn
    130                 135                 140

Cys Thr Ala Leu Gly Leu Ala Leu Asn Val Pro Gly Ser Ser Ser His
145                 150                 155                 160

Asp Thr Leu Cys Thr Ser Cys Thr Gly Phe Pro Leu Ser Thr Arg Val
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Gln Leu Lys Leu Arg Arg Arg Leu Thr Glu Leu Leu Gly Ala Gln Asp
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Arg Cys Arg Thr Gly Phe Phe Ala His Ala Gly Phe Cys Leu Glu His
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Gln Asn Thr Gln Cys Gln Pro Cys Pro Pro Gly Thr Phe Ser Ala Ser
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Cys Arg Arg Asp Ser Pro Thr Thr Cys Gly Pro Cys Pro Pro Arg His
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Gly Thr Pro Ser Gln Asn Thr Gln Cys Gln Pro Cys Pro Pro Gly Thr
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We claim:

1. A method for treating a patient in need of FLINT polypeptide activity comprising administering to said patient an effective amount of a FLINT polypeptide.

2. The method of claim 1 wherein the FLINT polypeptide is selected from the group consisting of a FLINT polypeptide analog, a biologically active FLINT polypeptide fragment, a FLINT polypeptide fusion protein and a FLINT polypeptide isoform.

3. The method of claim 1 wherein the FLINT polypeptide activity is binding of FasL.

4. The method of claim 3 wherein the binding of FasL inhibits apoptosis.

5. The method of claim 4 wherein the apoptosis causes or exacerbates sepsis.

6. The method of claim 4 wherein the apoptosis causes or exacerbates ARDS.

7. The method of claim 1 wherein the FLINT polypeptide is selected from the group consisting of SEQ ID NO:2, SEQ ID NO.: 5, SEQ ID NO:6, SEQ. ID NO.:7, SEQ ID NO.:8, SEQ ID NO.:9, and SEQ ID NO.:10.

8. A method for modulating selected TNFR interactions with their respective TNFR ligands comprising administering an effective amount of FLINT polypeptide.

9. The method of claim 8 wherein the TNFR family ligands are selected from the group consisting of FasL and LIGHT.

10. A method for modulating selected TNFR interactions with their respective TNFR family ligands *in vitro* comprising administering an effective amount FLINT polypeptide.

11. A method for modulating selected TNFR interactions with their respective TNFR family ligands *ex vivo* comprising administering an effective amount of FLINT polypeptide.

12. The method of claim 10 or 11 wherein the TNFR family ligands are selected from the group consisting of FasL and LIGHT.

13. A method for modulating FasL mediated apoptosis *in vitro* comprising providing a sample containing cells which undergo FasL mediated apoptosis and contacting said sample with an effective amount of FLINT polypeptide.

14. The method of claim 8 wherein the modulation is downregulation of FasL mediated apoptosis.

15. The method of claim 8 wherein the modulation is downregulation FasL mediated inflammation.

16. The method of claim 15 wherein the inflammation is massive neutrophil infiltration.

17. The method of claim 13 wherein the selected TNFR interactions with their respective TNFR ligands occurs in cells selected from the group consisting of T cells, myocytes, renal tubule epithelial cells (RTC), neutrophils, neurons, thyrocytes, stroma cells, acinar cells, Sertoli cells, macrophages, hepatocytes, and cells of the kidney cortex.

18. The method of claim 17 wherein said T cells are activated.

19. A method for treating conditions in a mammal which conditions are caused or exacerbated by FasL mediated apoptosis comprising identifying a mammal suffering from a condition caused or exacerbated by FasL mediated apoptosis and administering an effective amount of FLINT polypeptide.

20. The method of claim 19 wherein FLINT polypeptide is selected from the group consisting of a FLINT polypeptide

analog, a biologically active FLINT polypeptide fragment, a FLINT polypeptide fusion protein or a FLINT polypeptide isoform.

21. The method of claim 19 wherein said conditions are ARDS, sepsis, multiple organ failure and other conditions caused by massive apoptosis.

22. A method for treating conditions in a mammal which conditions are caused or exacerbated by FasL mediated inflammation comprising identifying a mammal suffering from a condition caused or exacerbated by FasL mediated inflammation and administering an effective amount of FLINT polypeptide.

23. The method of claim 22 wherein said conditions are ARDS, sepsis, multiple organ failure and other disease conditions exacerbated by massive neutrophil infiltration.

24. The method of claim 22 wherein the FLINT polypeptide is selected from the group consisting of a FLINT polypeptide analog, a biologically active FLINT polypeptide fragment, a FLINT polypeptide fusion protein or a FLINT polypeptide isoform.

25. The method of claims 1, 19, 20 or 22 further comprising administering antiinflammatory drugs or steroids.

26. The method of claims 1, 19, 20 or 22 wherein the FLINT polypeptide is administered in a single dose.

25. The method of claims 1, 19, 20 or 22 wherein the FLINT polypeptide is administered in a multiple doses.

ABSTRACT

METHOD OF TREATING ACUTE RESPIRATORY DISTRESS SYNDROME (ARDS) USING FLINT POLYPEPTIDES

Methods of preventing or treating conditions caused or exacerbated by inflammation and/or apoptosis by administering FLINT polypeptides are disclosed. Of particular importance are treatment of inflammation and/or apoptosis induced by Fas ligand (FasL) and Fas receptor (Fas) binding by administering FLINT polypeptides. Also disclosed are methods of treating ARDS, including ARDS correlated with sepsis. More generally, methods for preventing or treating disease by modulating interactions and/or binding between members of the TNFR superfamily and their respective ligands are disclosed.